THE UNIVERSITY OF ALBERTA

MICROBIOLOGICAL ASPECTS OF HIGH TEMPERATURE BEEF AGING

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled MICROBIOLOGICAL ASPECTS OF HIGH TEMPERATURE BEEF AGING submitted by Malcolm Sterling McDonald in partial fulfilment of the requirements for the degree of Master of Science.

A B S T R A C T

Beef is usually aged at approximately 2^{O} for periods ranging from 7 to 14 days. Aging at higher temperatures does allow for more rapid tenderization. Some of the microbiological aspects of high temperature beef aging were examined in this study.

Temperatures of 12.5^{O} and 15^{O} allowed for too rapid microbial development to be practical for use in aging beef. Spoilage was not as rapid at lower temperatures (10^{O}).

Relative humidity has an effect on the rate of microbial development when beef is aged at high temperatures. The counts/cm^2 of meat surface were higher at 95% relative humidity than at 85% or 75% when the storage temperatures were 10^{O} or 12.5^{O}. This effect was not evident at lower temperatures (4.4^{O} or 2^{O}). Low humidity levels (below 70%) did not markedly inhibit microbial development. A relative humidity of approximately 85% is recommended for aging beef.

Inoculated Salmonella typhimurium grew on meat stored at 10^{O} and 15^{O}. The rate of growth was less at 10^{O}. Salmonellae were not isolated from the meat samples or carcass swabs examined. This pathogen was thought not to be prevalent in the packinghouses examined.

Naturally occuring spoilage micro-organisms grew rapidly at the higher temperatures (10^{O} and 12.5^{O}) used for aging. Attempts to reduce the rate of spoilage by treating meat surfaces with chlorine or hot water sprays showed no clear cut advantage under the conditions used.

ACKNOWLEDGEMENT

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TABLE OF CONTENTS

L I S T O F T A B L E S

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF FIGURES (continued)

LIST OF APPENDICES (continued)

INTRODUCTION AND LITERATURE REVIEW

Current statistics indicate that Canadians consume more beef than any other meat product (Canada Year Book 1970-71). One of the most important quality characteristics of beef is tenderness (Joseph, 1970). Meat is usually tenderized by aging the carcasses at low temperatures (2^{o} to 5^{o}) for periods ranging from seven to fourteen days (Joseph, 1970; Miller, 1951). The temperatures of aging are kept low to inhibit microbial growth and the relative humidity is kept high to prevent excessive weight loss. If a process could be devised that would allow for shortening of aging time or allow all the various cuts to develop the same degree of tenderness, this would be of considerable economic importance to the meat packing industry (Joseph, 1970).

The exact mechanism of tenderization is not fully understood but it is known to involve enzyme action (Miller, 1951). General principles would therefore indicate that beef aged at 20^{o} should have the same degree of tenderness in one quarter of the time necessary at 0^{o}, since enzyme rate doubles for each 10^{o} rise (Joseph, 1970). Parrish et al. (1969) reported that aging at various temperatures showed no difference in tenderness development. They concluded that tenderization must take place early and the aging time must not effect it. However, they used young cattle (16 months) in their experiments and so the test animals might have had a higher than average tenderness as a group. Meat from the same muscle tested at the same time post-mortem is always tougher

in an older animal (Joseph, 1970). Parrish et al. (1969) also stated that other authors had reported findings contrary to theirs. Busch et al. (1967) stated that differences in tenderness can be related to the degree of muscle contraction. They showed that contraction was less at 16° than at 2° so that aging beef at the higher temperature should produce a more tender product. Wilson et al. (1960) also stated that higher temperature aging resulted in more tender beef.

Rey et al. (1970) reported experiments in which beef carcasses were aged at elevated temperatures. Nineteen cattle of sixteen months of age were slaughtered. One half of each carcass was aged continuously at 2° for 4 or 11 days. The other halves were aged either at 16° or 22° for one day and then at 2° for three days or at 16° or 22° for two days and then at 2° for two days. The meat was then cut, packaged, and stored at 5° to simulate retail storage conditions. Samples were tested for tenderness and for spoilage by microbiological plate counts at the end of the retail storage. They found that aging continuously at low temperature produced cuts of better keeping quality. However, if the aging time at 2° was extended to 11 days to give tenderness equivalent to the cuts aged at high temperature the bacterial counts were higher and the spoilage more rapid during retail storage. These authors also reported that salmonellae were not isolated from any of the carcasses. This work was carried out under laboratory conditions which were probably more sanitary than would be encountered in a large commercial operation. For this reason the authors suggest that their bacteriological counts might be better than those expected under more practical conditions. The major difficulty encountered when aging meat at elevated temperatures is spoilage.

With carcasses aged under traditional conditions, spoilage is normally a surface phenomenon (Elliott and Michener, 1965). Jay (1966) showed that the predominant microflora on aged beef were Gram-negative facultatively psychrophilic rods. Psychrophiles are defined as organisms which grow well at 1° (Barnes and Impey, 1968). Rose (1968) defined psychrophilic organisms as those which continue chemical reactions until the medium in which they are suspended freezes. Therefore spoilage can possibly result from psychrophilic enzyme activity where actual microbial growth is not evident (Elliott and Michener, 1965). The organisms associated with the spoilage of meat at low temperatures are predominantly species of Achromobacter or Pseudomonas (Barnes and Melton, 1971: Dainty, 1971). Aging at low temperatures inhibits the growth of food pathogens such as salmonellae because this pathogen may multiply very slowly at 10° and not at all at 5° or below (Salisbury and Crampton, 1960). Aging at higher temperatures increases the risk of growth of these pathogenic mesophiles. Spoilage organisms such as pseudomonads can also be a problem because their optimum growth temperatures are often in the 15°-25° range (Elliott and Michener, 1965). Ayres (1960) reported that spoilage organisms grew well at 5°, 15° and 25° on surfaces of various beef cuts.

The major concern with aging at elevated temperatures is to limit microbial growth and the resultant spoilage. Attempts have been made to limit the availability of water and thus prevent growth of micro-organisms. In Britain, packinghouse workers used cloths to wipe carcasses dry (Dainty, 1971). Government action was taken to prohibit this practice because it spread contamination from dirty to clean areas as well as from carcass to carcass. High relative humidities maintained

during aging also tend to promote microbiological growth (Joseph, 1970). Maintainance of relative humidity much below 80% tends to cause uneconomical weight loss (Dainty, 1971; Elliott and Michener, 1965). Ayres (1955) also suggested that moisture from the interior of the meat stored at low humidity diffuses to the surface where it is available for micro-organisms. Thus lowering the relative humidity does not seem practicable for controlling bacterial spoilage on meat aged at high temperatures.

The initial level of contamination on the meat surface is important in determining the rate of meat spoilage. The greater the initial load, the more rapidly spoilage will result (Elliott and Michener, 1965). Most of the bacterial load found on carcasses is derived from either the hides or the gut contents. Attempts have therefore been made either to remove the micro-organisms or to prevent their growth. Antibiotics and irradiation are two methods that have been used in attempts to control spoilage. The major meat spoilage organisms are among the most sensitive of all micro-organisms to irradiation (Wolin et al., 1957). However, unless the irradiation treatments are done at freezing temperatures, undesirable flavour changes result (Dainty, 1971). Antibiotic treatment has been shown to extend the shelflife of beef held at refrigerated temperatures for two days (Stringer and Nauman, 1966). Niven and Chesbro (1957) experimented with a combined antibiotic and irradiation process. In their work meat was treated with a low dose of irradiation (10^5 rep) as well as being dipped in a solution of the antibiotic oxytetracycline (10 ppm). This combined treatment extended the shelflife of the meat from 11 to 19 days. The authors found that if the meat storage temperatures were above the normal refrigerated temperatures (2°) the effect was lost

and rapid spoilage resulted.

Antibiotic treatment also presents the problem of selection of resistant forms of micro-organisms (Niven and Chesbro, 1957). Antibiotic resistant bacteria have been reported to grow more rapidly on treated meat because the competitive non-resistant forms have been eliminated (Hobbs and Wilson, 1959). Yeasts and molds are usually more resistant to both antibiotics and irradiation and often become a problem when comprtitive bacterial populations have been eliminated (Elliott and Michener, 1965). At present antibiotic and gamma irradiation treatments are not permitted in North America to control spoilage in fresh meats. The food industry tends to discourage use of processes which involve food additives such as antibiotics (Rey et al., 1970).

Pseudomonad spoilage organisms are sensetive to high carbon dioxide levels (Elliott and Michener, 1965). Although they are predominantly strict aerobes, growth has been recorded at oxygen levels as low as 1% (Joseph, 1970). Some techniques are being developed by Australian workers in which small cuts are aged in gas-permeable moisture proof films (Joseph, 1970). The carbon dioxide level is raised to 25% but the oxygen level is not allowed to fall below 15% so as to prevent metmyoglobin formation and the resultant meat discolouration. This technique of meat aging is useful in controlling the growth of strict aerobes but facultatively anaerobic micro-organisms such as lactobacilli soon take over and become the predominant spoilage forms (Bouton, 1968). These processes need more development before they can be used in high temperature aging treatments. Care must be taken to insure that the micro-organisms that are provided with more favourable growth conditions do not cause a

different type of spoilage or actually produce a public health hazard.

Ultraviolet light has been used to some extent to control surface growth on carcasses (Miller, 1951). Storage rooms fitted with these lights help prevent surface growth when they are used on a continuous basis. There is some difficulty in arranging the carcasses in a way that insures exposure of all surfaces to the light source so that both external and internal contamination is controlled. For this reason ultraviolet light has only limited usefulness in controlling the growth of micro-organisms on carcasses.

Meat reseachers at the Meat Research Institute, Bristol, reported that spraying with hot water helped to reduce counts on the surface of carcasses (Report, 1970). They found that spraying carcasses with water in the 75°-90° temperature range reduced surface counts by as much as 80%. They also stated that sprays containing high concentrations of chlorine effected reductions of surface counts of bacteria up to 99%. There seems therefore to be some possibility of effectively reducing surface counts on carcasses by using a spray technique but further research appears necessary to determine the most suitable temperature and the chlorine concentration to be used. The effect of water that remains on the carcass after spraying is also being studies at Bristol.

Aging beef at high temperatures can result in the growth of micro-organisms of public health significance. Since the microflora of aged beef is known to be predominantly Gram-negative rods (Jay, 1966) it would seem logical that the presence of Salmonella might present some problems. Under normal aging conditions (less than 5°) salmonellae do not grow actively (Salisbury and Crampton, 1960). However under high

temperature aging conditions there is an increased risk of rapid growth of these food pathogens. Weissman and Carpenter (1968) reported that 75% of the beef carcasses examined in their study in the U. S. A. were positive for salmonellae. Hobbs and Wilson (1959) state that Salmonella typhimurium is the commonest cause of Salmonellosis in the United Kingdom and that cattle are regarded as the largest animal source of this micro-organism. As meat surfaces seem to be more favourable toward the development of Gram-negative rather than Gram-positive bacteria, it seems probably that salmonellae could cause a health hazard in high temperature aging of beef.

Two approaches could be used to develope a method for high temperature aging of beef. These are tenderness and microbiological testing. The microbiological approach is indirect and allows the researcher to eliminate methods of aging that would be unsafe from a public health viewpoint. Tenderness is usually evaluated by the use of laboratory equipment such as the Cramer Shear Press or the Texturometer. To obtain reliable results from this type of experimentation many replications and statistical evaluations are required. Thus tenderness testing tends to be a costly and tedious procedure. In this work an attempt was made to determine conditions that would be hazardous from a public health viewpoint. By determining microbiologically unacceptable conditions some unnecessary effort and duplication of work could be avoided.

MATERIALS AND METHODS

Treatment of Samples

The chuck steak used in the preliminary experiments was obtained from a local supermarket and stored in the laboratory at 2^{o} until used. Freshly butchered and shrouded flank steak was sampled at a local packing-house. Sterile knifes and tongs were used to remove sections of the meat. The meat was placed in sterile plastic bags and stored at 2^{o} until used. Meat was usually obtained 3 hr. before the beginning of the aging period. The sections of meat were cut into small pieces of approximately 16 cm^{2} with sterile scalpels in preparation for inoculation or storage.

Samples of meat to be aged were placed in one half of a sterile plastic Petri dish. These were placed in large desiccators which contained a saturated salt solution in the bottom section to control relative humidity. Relative humidities (RH) of 95, 85, 75 and 40% were obtained by using saturated solutions of $NaSO_4.H_2O$, KBr, $NaClO_3$ and $CaCl_2.H_2O$ (Spencer, 1926, Wexler and Hasegawa, 1954). Relative humidity of less than 1% was obtained by using $CaCl_2.H_2O$ as a desiccant. The desiccators were stored in a walk-in environmental room at the desired storage temperature which varied by $\pm 1^{o}$.

Samples were inoculated with a culture of Salmonella typhimurium ATCC 13311. The inoculum was obtained by growing the culture in 100 ml of Nutrient Broth in 250 ml erlenmeyer flasks on a Gyrotory Shaker Incubator (Model G-25) at 160 rpm and 37^{o}. After the first 18 hr. of growth, 1 ml of the culture was transferred to 100 ml of fresh broth and

incubated for 14-18 hr. One ml of this culture was transferred to 100 ml of broth and after 14-18 hr. incubation this culture was used as the inoculum. A 10^{-1} dilution was prepared in peptone water (see later) and 0.1 ml of this was pipetted on the 16 cm^2 meat surface. The inoculum was spread over the entire surface using a flamed bent glass rod.

Bacteriological Sampling

Inoculated samples were used in preliminary studies to test the effectiveness of sampling procedures. Thin slices of surface were removed, weighed, and counts reported /g. Known surface areas were cut with a metal cutter and entire surface membranes were removed and counts reported /cm^2. As reproducible results were not obtained with these techniques, swabbing was used throughout most of the experiments. Sterilized cotton swabs were moistened in a peptone dilution blank and an area of 9 cm^2 was swabbed. The swab was returned to the dilution blank, rinsed and the area reswabbed. Sterile scissors were used to cut the bottom half of the swab off into the original dilution blank which was vibrated for 20 sec. on a Vortex mixer and used to make the necessary dilutions.

The swabbing technique was used in both the laboratory work and in the examination of carcasses in industrial aging rooms. Patterson (1971) reported that one swabbing removed approximately 50% of the organisms present on a surface. An error of this magnitude would have little significance when comparing counts of eg. 10^4 with $10^8/cm^2$. It was considered that the simplicity of this method and its application to both laboratory and industrial situations out-weighed the disadvantages.

Plating Method

A surface plating technique was used throughout the work. This was necessary in the preliminary work because only surface colonies of Salmonella spp. gave typical reactions on the selective media used. Graduated Pasteur pipettes were used to apply the sample dilutions to the agar surfaces. The bottom of the plates were marked with a wax pencil to divide the plate into two sections. Duplicate drops were each spread over one half of the plate with a sterile bent metal wire. One pipette was used to plate all the dilutions from the same sample starting at the highest and working towards the lowest dilution. The wires were flamed between spreading inocula on different types of media. With each experiment sterile pipettes and wires were used to dispense a sterile diluent as a control. Three dilutions were plated for each sample and plates with colony counts between 15-150/drop were used where possible to calculate the numbers/cm^2. The plating method used was similar to that described by Clark (1971).

The radius of the tips of commercially produced Pasteur pipettes varied widely. As stated by Davis (1971) the tip radius is one of the factors that governs the size of a drop delivered by a pipette. The tip radius was made constant by cutting the pipettes off to a standard size that delivered 33 drops/ml of peptone water. Pipettes were placed in a standard sized hole in a metal block, marked with a file, and broken off to the correct size. Other standard reusable and disposible glass bacteriological pipettes were also used.

Peptone water (0.1%), peptone water with $MgSO_4$ (10^{-4}M) potassium phosphate (pH 7) and Ringer's solution (¼ strength) were tested for

suitability as a diluent. Dilutions of a culture of Salmonella typhimurium grown over three transfers as described previously, were prepared in each of the four diluents. Each was plated immediately, then stored at 24° and plated at hourly intervals over a 7 hr. period. Fig. 1 shows that the difference in counts between the two peptone solutions and the Ringer's solution were negligible, whereas the organisms suspended in phosphate buffer died off in a few hours. Straka and Stokes (1957) reported that 80% of the natural flora of poultry pies was destroyed within 1 hour when phosphate buffer was used as the diluent. They recommended the use of (0.1%) peptone water in the dilution of food products for microbiological examination. As peptone water (0.1%) was the simplest diluent to prepare it was used as the diluent throughout this work. Tubes of diluent used in swabbing meat that had been treated with water sprays contained 0.002% (w/w) sodium thiosulphate to neutralize any residual chlorine. (Harrigan and McCance, 1966).

Media was prepared according to the manufacturers' instructions and poured in a laminar flow hood. The lids of the plates were left partially off for 20 min. during solidification to prevent excess condensation. Solidified plates were stored on a laboratory bench for at least one day before being used. Plates that were stored for longer than five days were placed in plastic bags to prevent excess drying. Disposable plastic Petri dishes were used throughout.

Bacteriological Differentiation

Total Plate Colony Count. Counts for mesophilic organisms were done on Plate Count Agar (PCA) (Difco). Plates were incubated at 37° for two days. This count is referred to as TPC.

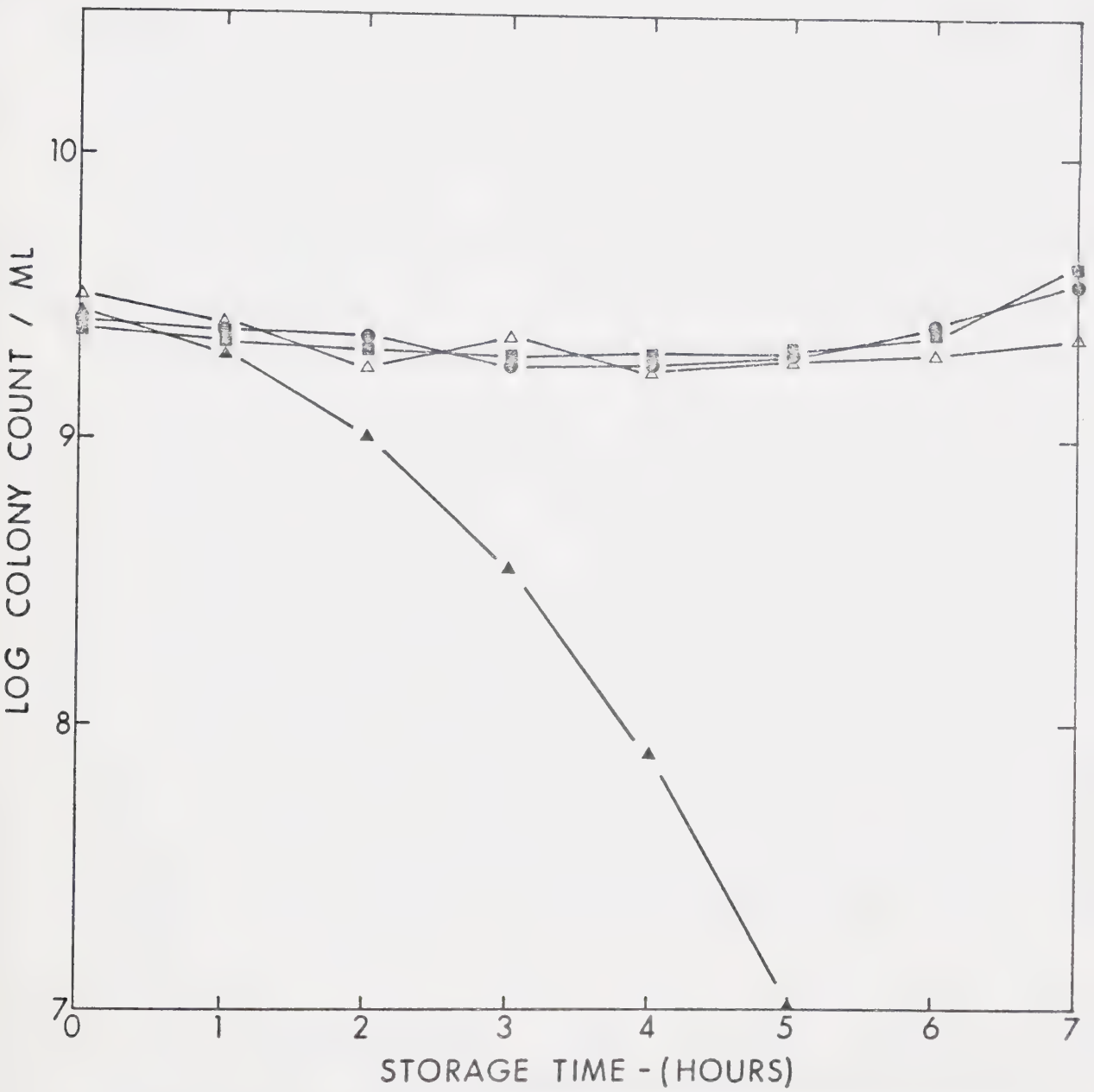

Fig. 1 The effect of various diluents on the survival of a diluted Salmonella typhimurium culture stored at 24°. Count at 37° on PCA: ● peptone water (0.1%); ■ peptone water (0.1%) with $MgSO_4$ (10^{-4} M); ▲ potassium phosphate buffer (pH 7); △ Ringer's solution (1/4 strength). See Appendix I

Pseudomonad Count. The organisms important in the spoilage of meat are known to be predominantly members of the Pseudomonas genus (Barnes and Melton, 1971). These organisms are described by Elliott and Michener (1965) as having optimal growth temperatures between 20° and 30°. Barnes and Melton (1971) used an incubation temperature of 20° for these micro-organisms. The most convenient temperature available during this work was 24° or the temperature of an air conditioned laboratory. As can be seem by comparing Fig. 22 with 23 and 25 with 26 the counts at 24° for two days were similar to those at 10° for ten days which was the temperature used by Rey et al. (1970). The B medium of King et al. (1954) is not selective but was originally designed to inhance pigment production by pseudomonads. These organisms grow readily on this medium and it was used to estimate the numbers of pseudomonad spoilage organisms present on meat surfaces in this study. King et al. (1954) recommended preparation of a stock solution of 15 g/100 ml of each of K_2HPO_4 and $MgSO_4.7H_2O$ but these salts formed a percipitate when mixed at that concentration. Separate stock solutions of 15 g/l. were maintained and addition of these to liter quantities of medium did not result in the percipitate formation. In this work the count on the B medium of King et al. (1954) at 24° for two days is referred to as the Pseudomonad Count (PC). However some of the preliminary pseudomonad counts were done on PCA at 10° for 10 days as used by Rey et al. (1970).

Salmonella Count. Brilliant Green Agar (BGA) (Difco) was used to determine the numbers of salmonella present. Pink-white opaque colonies surrounded by reddening of the medium were considered typical salmonellae. As reported by Georgala and Boothroyd (1965) this medium was found to be

less inhibitory than other selective media such as Salmonella-Shigella Agar (Difco). Suspicious colonies were transferred to Triple Sugar Iron Agar (TSIA) (Difco) for confirmation. A salmonella count was done on all samples throughout the work.

Coliform Count. Eosin Methylene Blue Agar (EMBA) (Difco) was used in each experiment. This medium was used because it is not markedly inhibitory and would show any heavy coliform contamination that occured in the meat being examined.

Salmonella Enrichment. A salmonella enrichment procedure similar to that recommended by U. S. D. H. E. W. (1964) was used for both meat samples and swabs. Ten ml of a 10^{-1} dilution of meat was added to 90 ml of Nutrient Broth (Difco) and incubated at 37^{o} for 14-18 hr. Swabs were treated similarly except that an equal volume of double strength Nutrient Broth was added to the dilution blank containing the swab. After the incubation period, 1 ml of the broth was transferred to 9 ml of Selenite Cysteine Broth (Difco) and to 9 ml of Tetrathionate Broth (Difco). These broths were incubated for 18-24 hr. and each was streaked on BGA. Typical salmonella colonies were transferred to (TSIA) tubes and positives were confirmed as described below.

Salmonella-Pseudomonas Differentiation. Pure cultures of Pseudomonas spp. streaked on BGA gave a reddening reaction similar to that of salmonellae. These organisms also gave an alkaline slant and acid butt on TSIA which is the typical salmonellae reaction, although these micro-organisms usually produce H_2S whereas the pseudomonads do not. To distinguish between these two micro-organisms the O-F Glucose Medium of Hugh and Leifson (1953) was used. Salmonellae can metabolize glucose

both oxidatively and fermentatively while pseudomonads have predominantly oxidative metabolism (Cowan and Steel, 1965). Therefore cultures that did not produce any acid reaction in the wax covered Hugh and Leifson tube were considered not to be salmonellae. After working with this test for several months it became unnecessary because pseudomonad colonies were easily identified as they tended to be more pigmented and raised than the salmonellae colonies.

Stock Cultures

Several stock cultures were maintained on Stock Culture Agar (Difco) enriched with 1% Yeast Extract (Difco). These cultures were used for comparisons on selective and differential media. The cultures were Salmonella typhimurium ATCC 13311, Pseudomonas fluorescens ATCC 17400, Escherichia coli B (Roth, 1969), two pigmented Pseudomonas spp. and two Achromobacter spp. The latter four cultures were obtained from Dr. D. S. Clark, National Research Council, Ottawa and had been isolated from meat.

Relative Humidity Measurement

Three methods were attempted to measure the relative humidity within the desiccators, however all were unsuccessful. A membrane type instrument that could fit into the jars was tested against accurate equipment in the university Botany Department and was found to be inaccurate. Therefore it was assumed that the relative humidities in the desiccators were close to those given in standard tables (Spencer, 1926, Wexler and Hasegawa, 1954). These tables indicated that there was little variation with small temperature differences (20°). The relative humidities given for saturated salt solutions at 20° and 25° were considered to be the same at the temperatures used in this study (2°, 4.4°, 10°, 12.5° and 15°).

Relative humidity in packinghouse aging rooms was measured with a Bendex Psychron psychrometer. This instrument (Model 566-3) has a battery operated fan which draws a standard volume of air/unit time past wet and dry bulb thermometers. The accuracy of the thermometers is ± 0.15° which could result in an RH difference of approximately 5%.

Chlorine Treatment

All chlorine solutions were used at 24°. Tap water was used as a control and had a residual chlorine level of 3 parts per million (ppm). A commercial laundry bleach (Javex) was diluted with tap water to prepare solutions of 20 and 500 ppm chlorine. Chlorine concentration was measured by the use of diethyl-p-phenylene diamine (DPD) comparator tablets in a Lovibond Comparator. The meat was held 4 in. from the nozzle of a pint sized, hand operated garden spray, and spraying was continued for 10 sec. After spraying, the samples were cut asceptically into small portions and placed in a desiccator for aging.

Hot Water Treatment

Tap water was used for hot water spraying through a garden hose nozzle adjusted to the spray position. The control spray was at normal tap temperature (18°-20°) and higher temperatures were achieved by allowing varying amounts of steam to enter the water flow. The highest temperature available using this equipment was 70°. Sections of meat were held 4 in. from the nozzle and spraying was continued for 10 sec. The meat was then cut into smaller portions and placed in the desiccators for aging. Samples tested on the first day in spraying experiments were stored at 2° for 15 min. before being swabbed.

RESULTS AND DISCUSSION

The initial experiments in this work were an attempt to study the growth of Salmonella typhimurium on meat surfaces. A cheap meat cut, chuck steak, was purchased at a local supermarket. The meat was cut into small pieces and half were inoculated with Salmonella typhimurium as described in the methods section. All samples were stored at 15° and 95% RH. The results of this test, which was done in duplicate, are shown in Figs. 2 and 3. These show the TPC at 37° and the salmonella count on chuck steak stored for one day. The uninoculated samples in Fig. 2 had a low initial count but this increased from 10^2 to $10^7/cm^2$ after only one day of storage at 15°. No salmonellae were isolated from uninoculated samples while both the TPC and the salmonella count on inoculated samples showed an increase. Results illustrated in Fig. 3 show similar trends although the initial uninoculated counts were higher. These samples were obviously spoiled because of the presence of strong odour and discolouration after one day of aging at 15°.

Fig. 4 represents the results from a similar experiment except that the storage temperature was 10°. After two days at this temperature the TPC and the salmonella count were similar to those on the samples stored at 15° for one day. Salmonella counts on inoculated samples increased from 10^6 to $10^8/cm^2$, thus there was some growth of this pathogen at the lower temperature. The samples in this experiment had spoiled after five days of aging.

Ayres (1960) considered that slime formation resulted when the

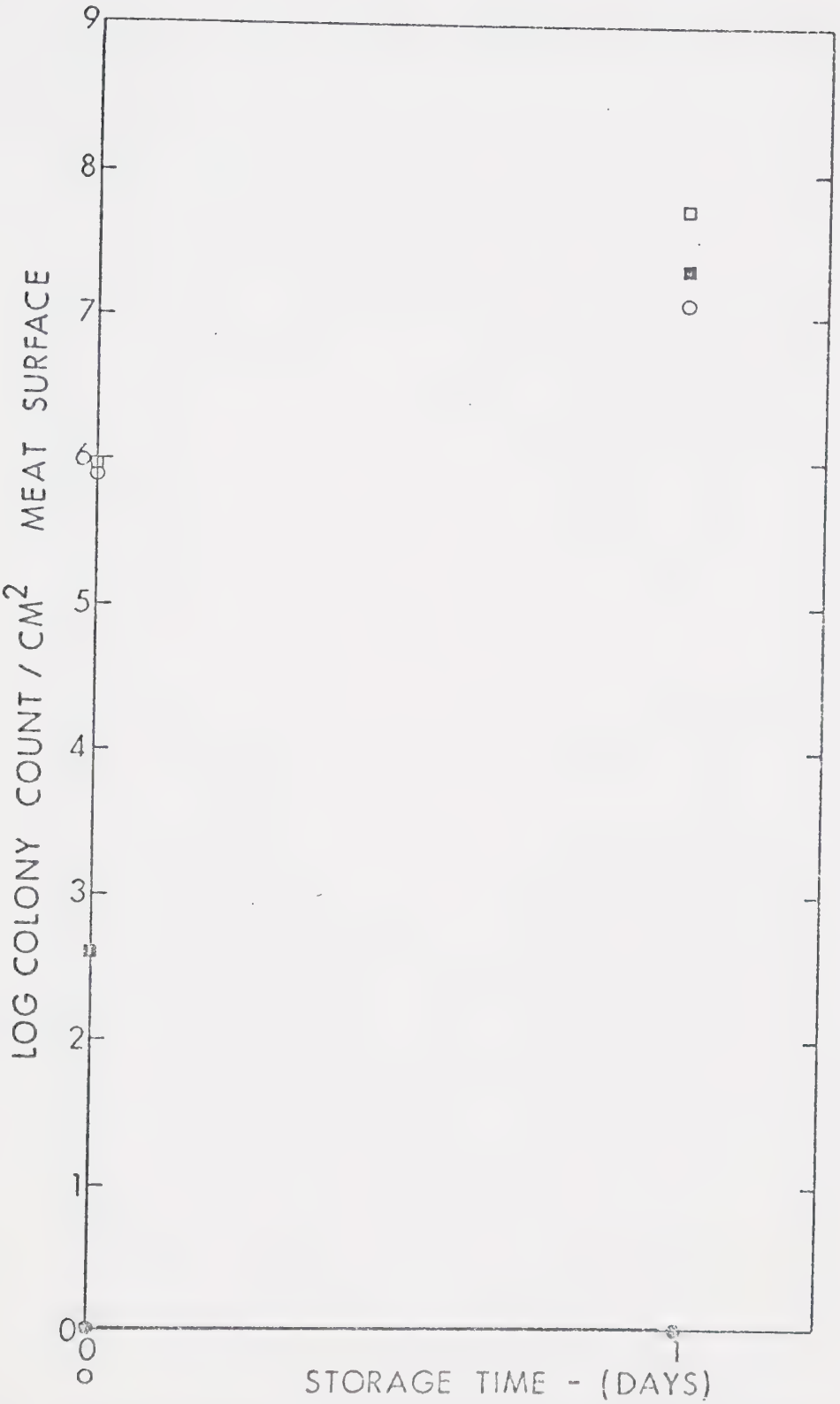

Fig. 2 The development of micro-organisms on the surface of chuck steak stored at 15° and 95% relative humidity with and without Salmonella typhimurium inoculation. Count on PCA: ▪ uninoculated; □ inoculated. Count on BGA: ● uninoculated; ○ inoculated. See Appendix II A.

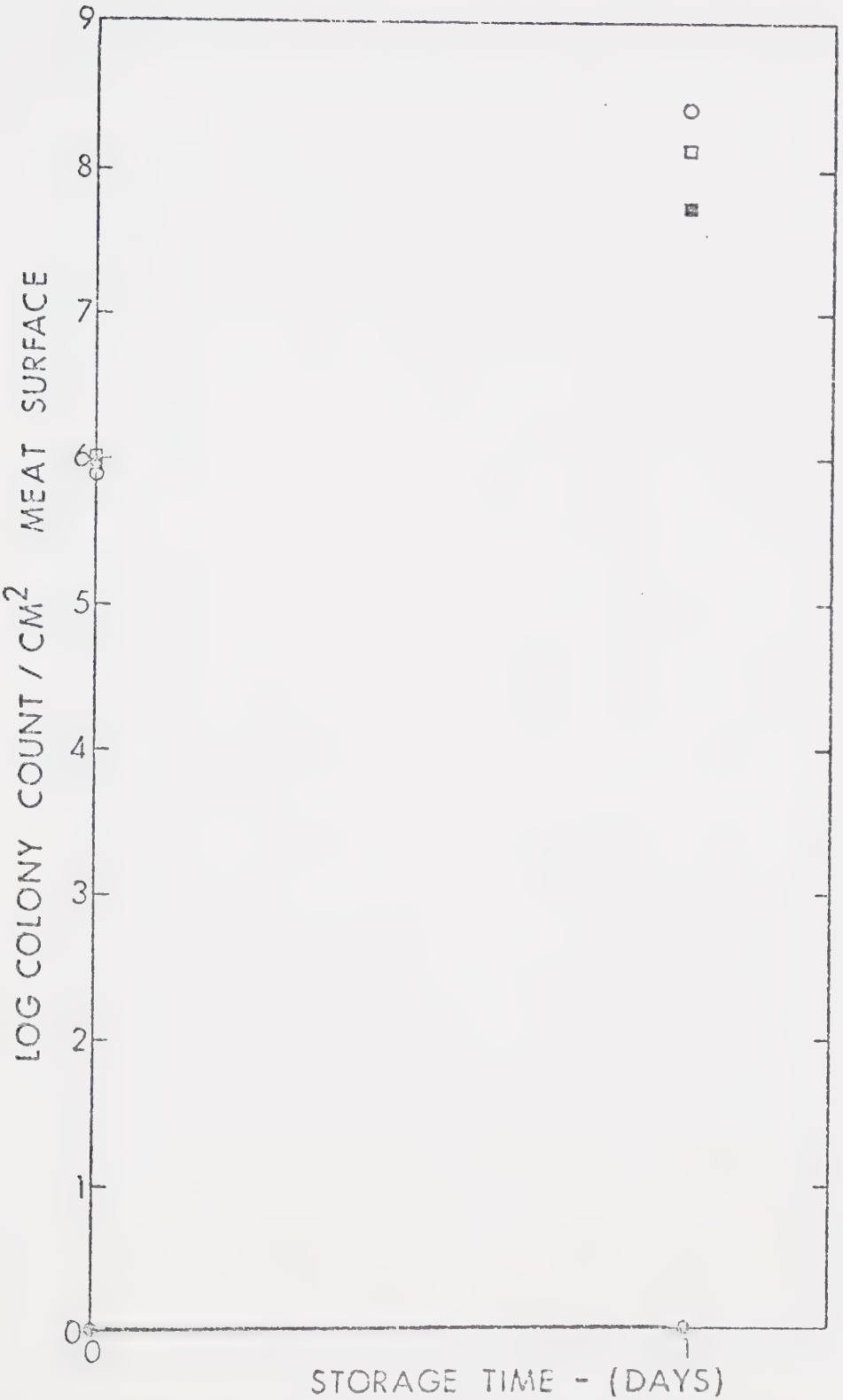

Fig. 3 The development of micro-organisms on the surface of chuck steak stored at 15° and 95% relative humidity with and without Salmonella typhimurium inoculation. Count on PCA: ■ uninoculated; □ inoculated. Count on BCA: ● uninoculated; ○ inoculated. See Appendix II B.

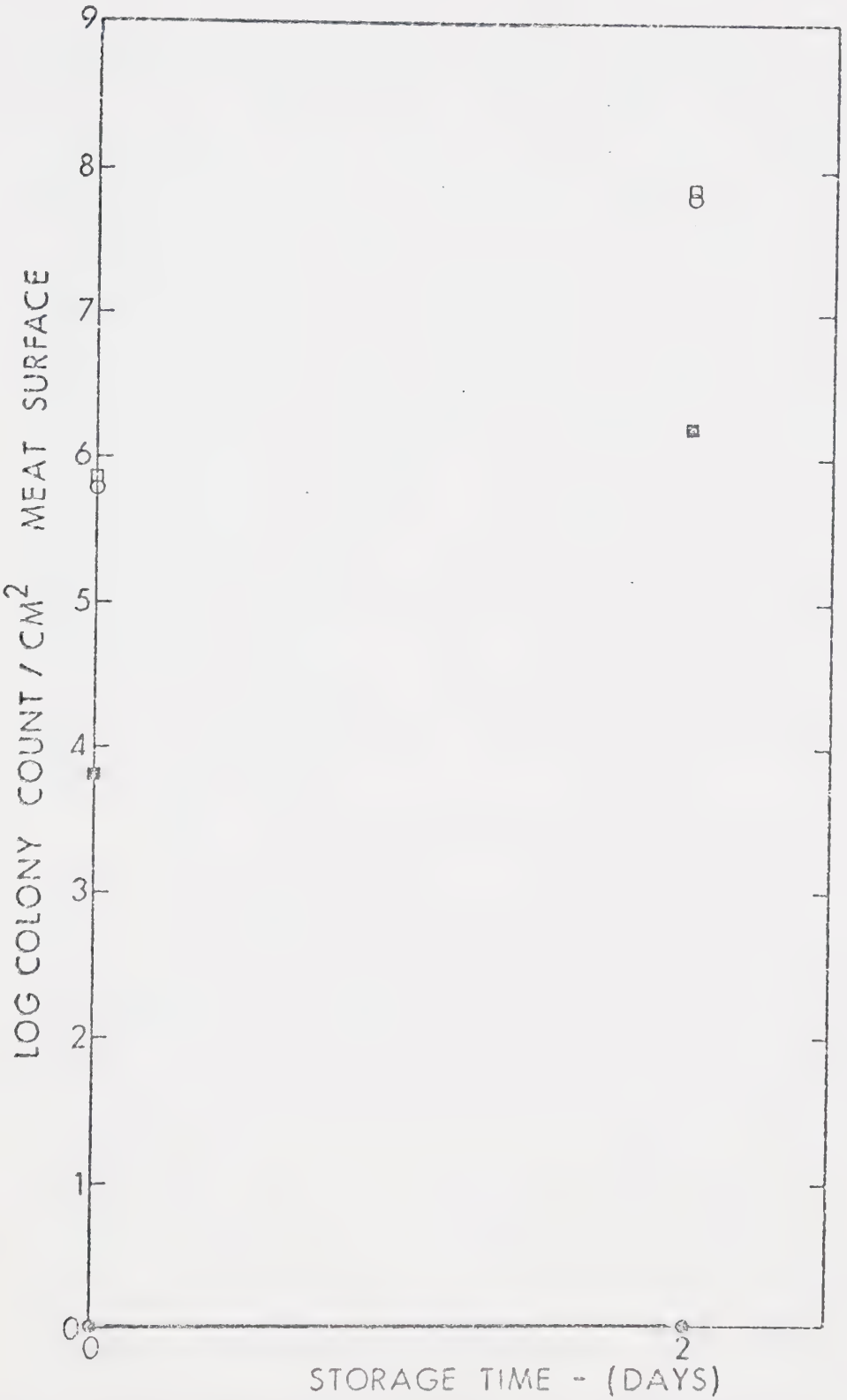

Fig. 4 The development of micro-organisms on the surface of chuck steak stored at 10° and 95% relative humidity with and without Salmonella typhimurium inoculation. Count on PCA: ■ uninoculated; □ inoculated. Count on BGA: ● uninoculated; ○ inoculated. See Appendix III.

surface count on meat exceeded $10^8/cm^2$. This limit was approached in the chuck steak stored at both 10^o and 15^o. The meat used in these preliminary experiments was probably at least 10 to 14 days old when brought to the laboratory. It would also have undergone more handling and therefore could have a higher bacterial load than would be found on carcasses undergoing normal aging. Cut surfaces were used and these are known to spoil more rapidly than the membrane or fat covered surfaces of a carcass. For these reasons it was considered desirable to repeat this experiment using an uncut surface from freshly killed animals. The meat was obtained from the flank of a carcass three hours post-slaughter. The internal membrane surface was used as the area for inoculation and sampling. The experiment was repeated at 10^o since the bacterial growth rate had been so high at 15^o.

Fig. 5 summarizes the TPC at 37^o and 10^o on flank steak aged at 10^o for four days. The initial counts showed that the meat had a high level of natural contamination. The organisms present must have been predominantly of the mesophilic type because micro-organisms capable of growth at 10^o were not detected initially on either the uninoculated or inoculated samples. After one day of storage the count at 10^o on uninoculated samples showed a large increase. At the end of the four day storage period all counts were in the range of 10^8-$10^9/cm^2$. In this experiment the development of salmonellae was greater than in other experiments. Although the development of salmonellae in this experiment was similar to that of pseudomonads, in none of the work here or in earlier experiments (not reported here) did the salmonellae outgrow the pseudomonads. Accordingly and to simplify the experimental procedure inoculation of

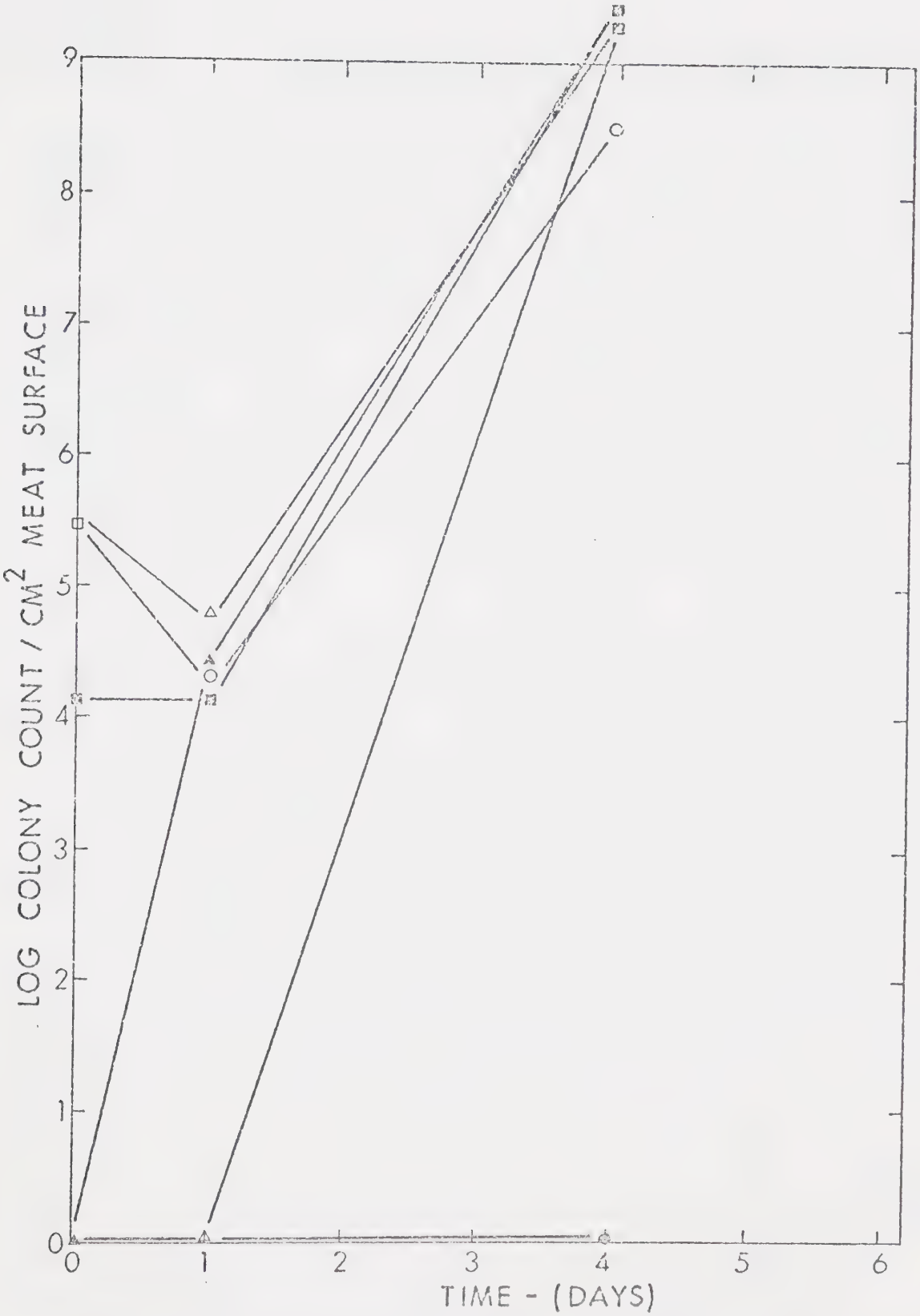

Fig. 5 The development of micro-organisms on the surface of flank steak obtained 3 hr. post-slaughter and stored at 10° and 95% relative humidity with and without Salmonella typhimurium inoculation. Count at 37° on PCA: ■ uninoculated; □ inoculated. Count at 10° on PCA: ▲ uninoculated; △ inoculated. Count on BGA: ● uninoculated; ○ inoculated. See Appendix IV.

meat surfaces with Salmonella typhimurium was later discontinued.

The results of this experiment were suprising because freshly slaughtered meat was not expected to spoil as rapidly as the already aged chuck steak. These samples spoiled as or more readily than the samples of chuck steak previously examined. The only process which was carried out under industrial conditions and not in the laboratory was shrouding. shrouding involves soaking a strong mesh cloth in water or a brine solution. This is then wrapped tightly around the carcass and pinned in place for the first 24 hr. of aging. The main object of the procedure is to firm up the fat by surface cooling and thus give the carcass a smooth finish. Also when the shroud is removed it takes with it some small surface veins and this helps to improve the carcass appearance. The appearance of the carcass has an influence on the official grading. The process of shrouding with wet cloths tends to decrease moisture loss and maintains a high moisture level at the surface of the carcass, which would encourage microbial growth rather than inhibit it. Since shrouding was the only process omitted in the laboratory work a further experiment was done to consider this aspect. This trial involved the use of two day old flank steak that had been shrouded for the initial 24 hr. Uninoculated and inoculated meat samples were stored at 10^{o} and examined at 0, 4 and 6 days. Counts at 10^{o} were initially low on both uninoculated and inoculated samples but increased rapidly after 4 days storage as illustrated in Fig. 6 with simultaneous spoilage. Surface growth was not less rapid on meat that had been shrouded. The carcass surfaces appeared to have less moisture than the meat surfaces used in laboratory tests.

The relative humidity (RH) used under laboratory storage conditions

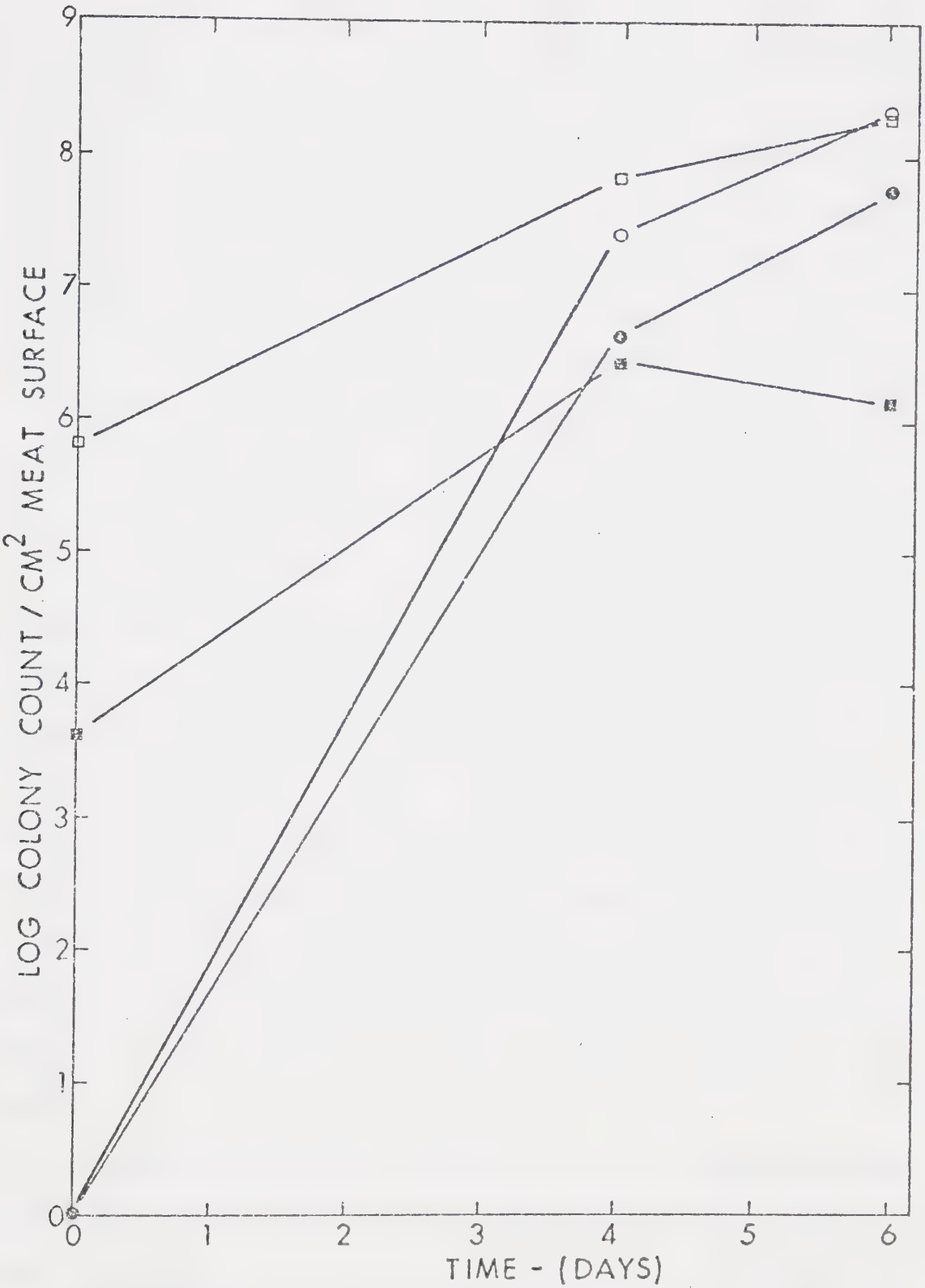

Fig. 6 The development of micro-organisms on the surface of flank steak obtained 2 days post-slaughter and stored at 10° and 95% relative humidity with and without Salmonella typhimurium inoculation. Count at 37° on PCA: ▣ uninoculated; □ inoculated. Count at 10° on PCA: ● uninoculated; ○ inoculated. See Appendix V.

was high (95%). This was thought to be close to the RH maintained in the local packinghouse storage rooms. The recommended level is between 85 and 90% (Joseph, 1970). Laboratory samples that had a TPC that exceeded $10^6/cm^2$ had a strong odour and were spoiled. This level of surface growth was detected within 2-4 days in most of the experiments done in the laboratory. Although the aging temperature used (10°) was higher than that used in the industry (2°), the counts reached $10^6/cm^2$ more rapidly than expected. The rapid spoilage in our experiments was not caused by handling in the laboratory but was considered to be due to the higher RH used in laboratory aging. Because of this, carcasses at a local packinghouse were examined by the swabbing technique to learn what level of microbial populations developed under commercial conditions. Initially various areas of freshly butchered and seven day old carcasses were swabbed on two occasions to determine if any areas had particularly high counts. As can be seen in Table 1 the TPCs at both 10° and 37° were lower in most cases on the older carcasses. None of the areas examined had extremely high counts and it seemed unlikely that there was growth on the carcasses stored under the conditions in the plant that was examined. It appeared that the major difference between laboratory and industrial aging, with the exception of temperature, might be a difference in RH.

The following phase of the work involved a study of the RHs and carcass surface contamination in aging rooms in three Edmonton packinghouses. Each plant was visited twice. Three swabs were taken from different areas of each of a total of 30 carcasses of various ages. These were plated for TPC at 37° and for pseudomonad count (PC) at 24°. The

Table 1 Swab examinations of different areas of fresh and aged beef carcasses.

Area Swabbed	Total Plate Count/cm^2 of Meat Surface			
	Day 0*		Day 7	
	37°	10°	37°	10°
Hindleg	70	5	16	3
Flank	8	3	30	3
Rib	162	640	14	<3
Back	459	459	19	<3
Hindleg	143	81	73	38
Flank	19	14	68	89
Rib	189	59	8	5
Back	181	116	62	122

* swabbed immediately after slaughter

results are summarized in Figs. 7-12. Figs. 7, 8 and 9 are the results from the TPCs for flank, middle rib and forward rib areas of the inner side of the carcass respectively. Figs. 10, 11 and 12 are the PCs for the same areas. The numerals plotted on the graphs indicate individual carcasses. These Figs. 7-12 illustrate that there can be a wide variation in counts between swabs from different areas on the same carcass and between similar areas on different carcasses of the same age.

The RH in the slaughterhouses were somewhat lower than had been expected. All measurements during this period were within the 70-80% range. Plants had no accurate methods of measuring RH, made no regular tests and made little or no effort to control RH. One plant opened steam valves to increase the humidity when it fell too low. When experimental measurements were continued at another plant over an extended period, the RH seemed to vary with the prevailing weather condition. On a normal clear day the RH was about 75% and on rainy days it was as high as 95%. However the RH was usually within the 70-80% range.

The lower RH that was prevalent in the packinghouse aging rooms may have contributed to the low rate of bacterial growth on carcass surfaces. Rey et al. (1970) reported counts not greater than $10^5/cm^2$ on carcasses that had been stored at 16° for two days and then at 5° for 3 days. The counts on our samples stored at 15^{0} exceeded this after only 1 day of storage. No direct comparison can be made between our laboratory aging results and the packinghouse swabbing results because of the difference in storage temperatures. Counts on commercially aged carcasses were generally within an acceptable range. Although this was expected because of the lower storage temperatures used, it was also thought that

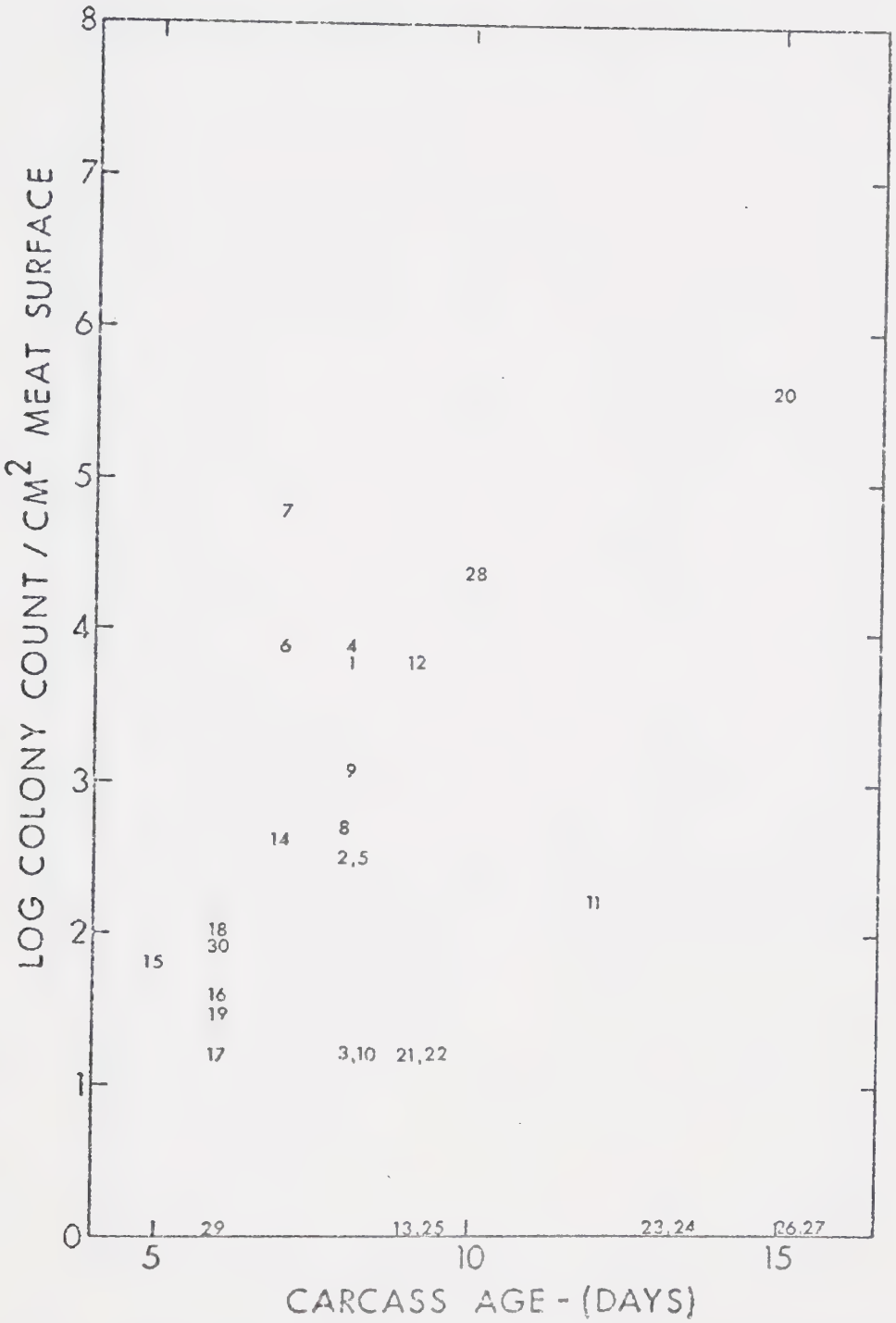

Fig. 7 The relationship of aging time to the numbers of micro-organisms on the flank area of beef carcasses stored under industrial conditions. Count at 37° on PCA. See Appendix VI.

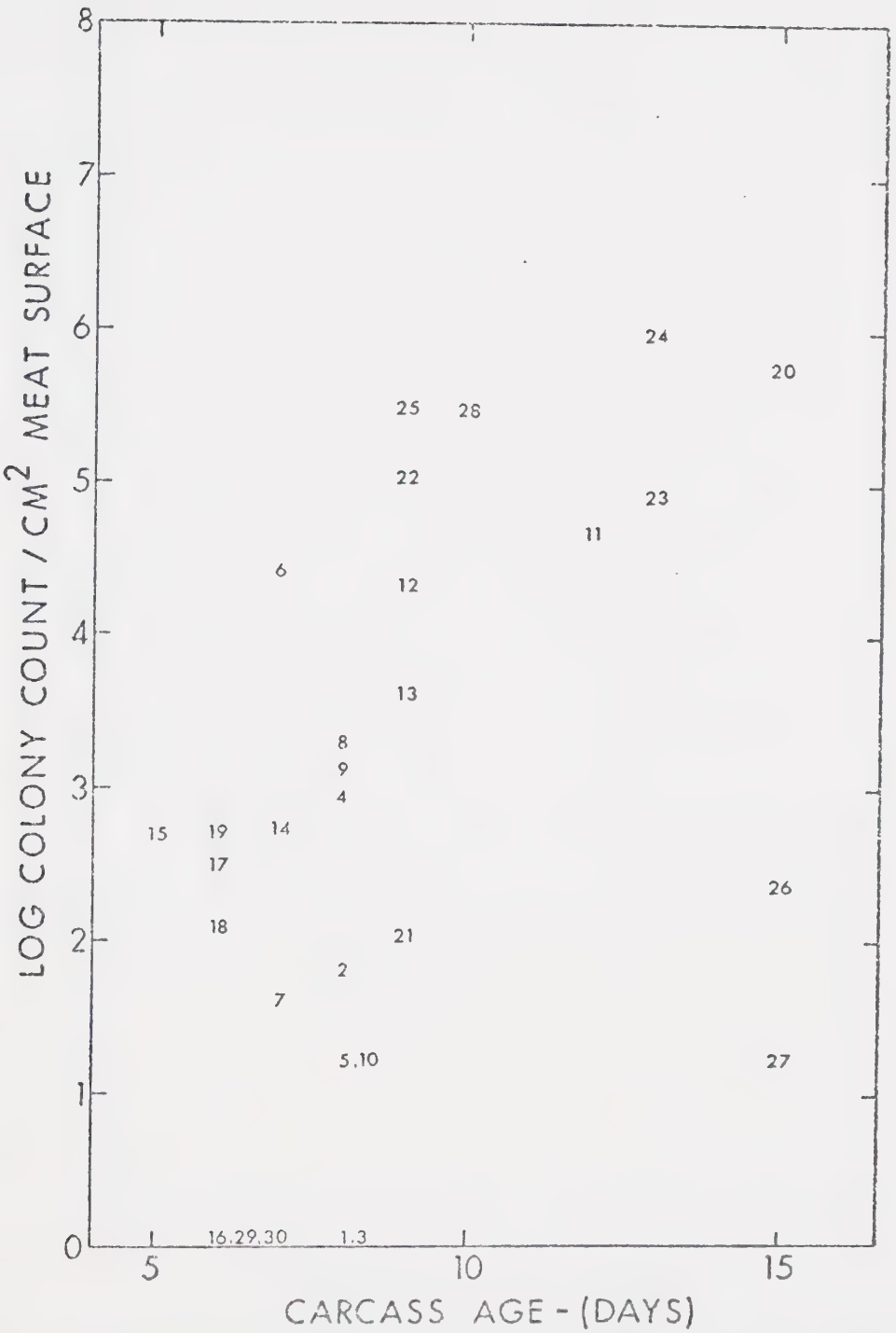

Fig. 8 The relationship of aging time to the numbers on micro-organisms on the mid-rib area of beef carcasses stored under industrial conditions. Count at 37° on PCA. See Appendix VI.

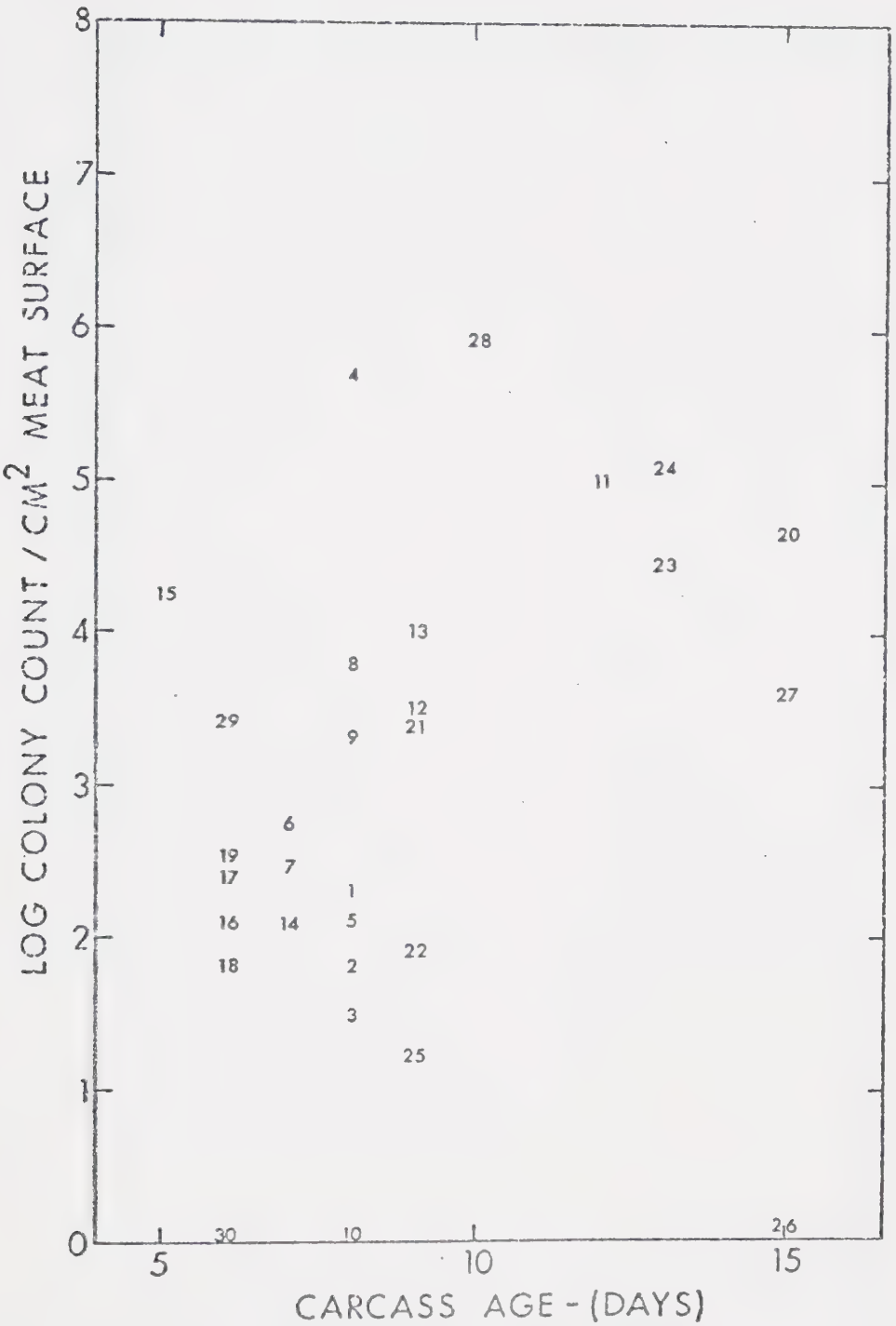

Fig. 9 The relationship of aging time to the numbers of micro-organisms on the forward rib area of beef carcasses stored under industrial conditions. Count at 37° on PCA. See Appendix VI.

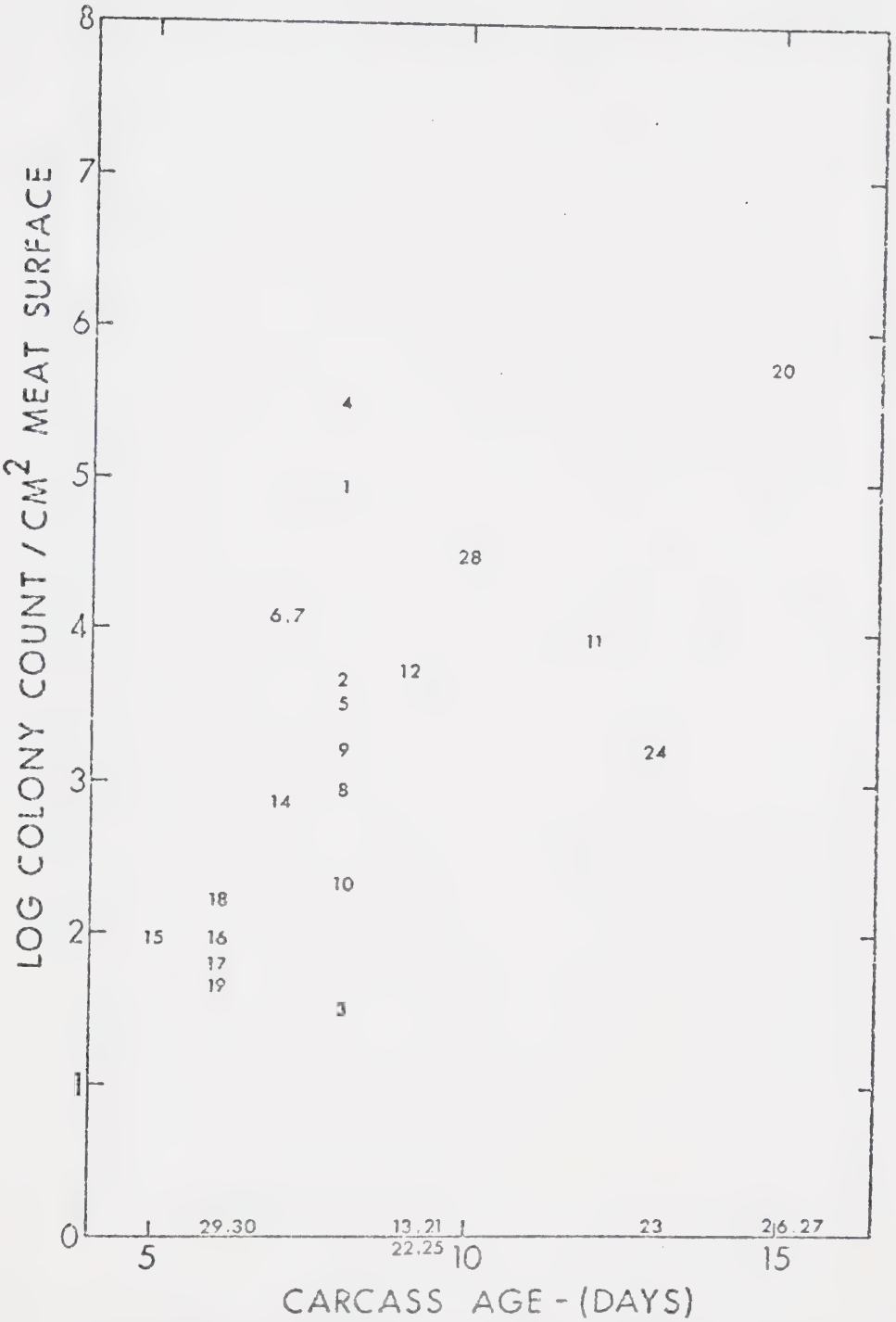

Fig. 10 The relationship of aging time to the numbers of micro-organisms on the flank area of beef carcasses stored under industrial conditions. Pseudomonad count on the B medium of King. See Appendix VI.

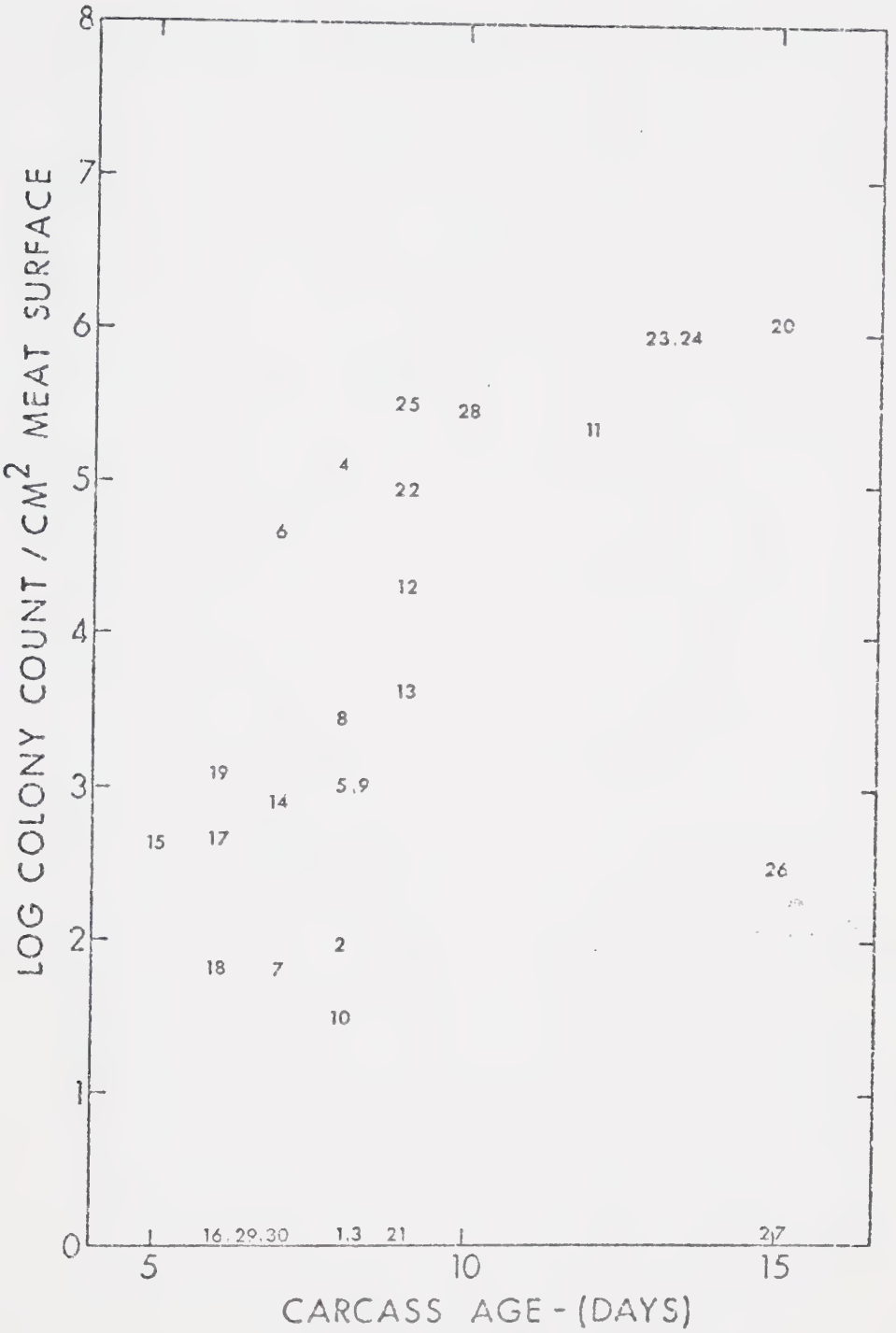

Fig. 11 The relationship of aging time to the numbers of micro-organisms on the mid-rib area of beef carcasses stored under industrial conditions. Pseudomonad count on the B medium of King. See Appendix VI.

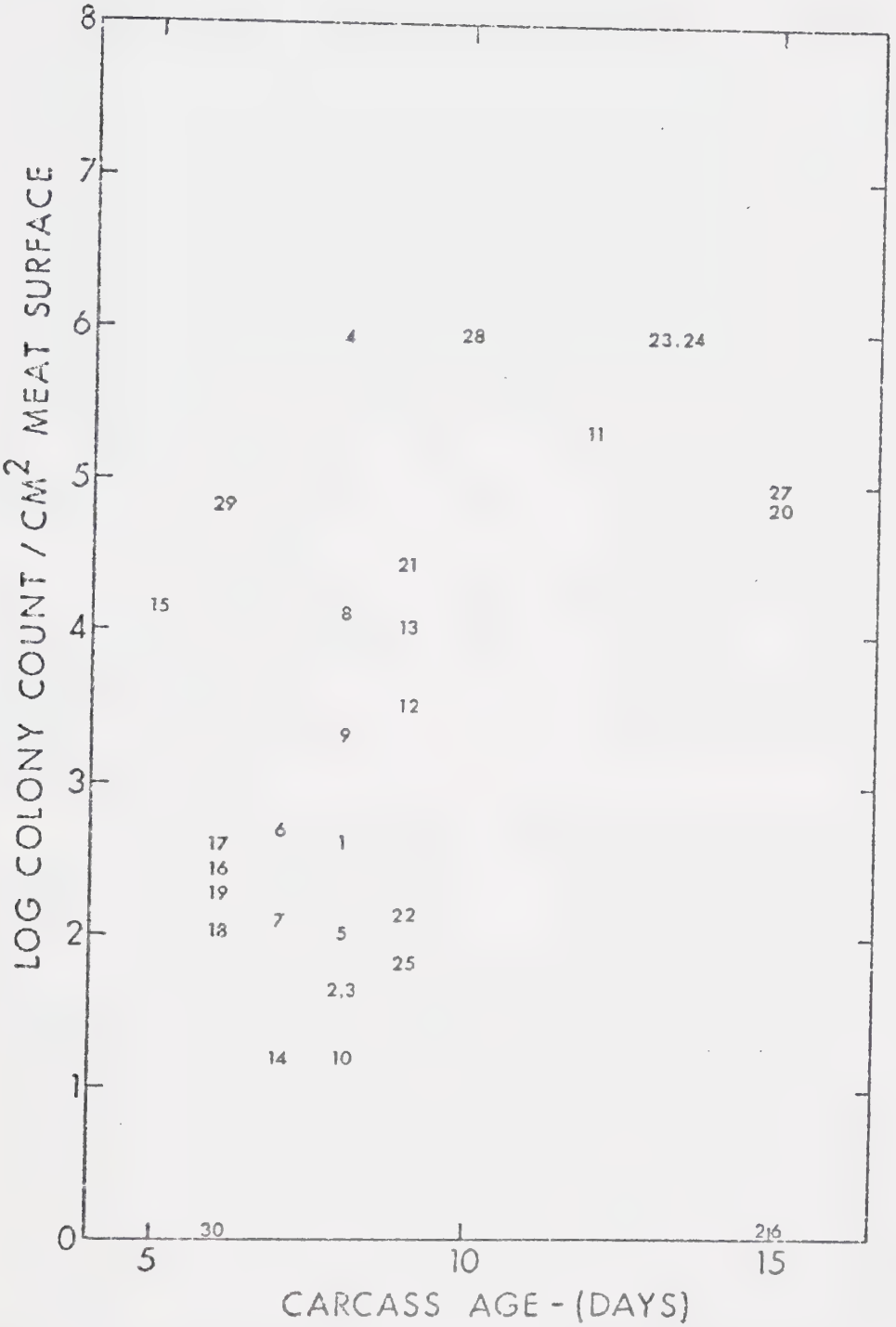

Fig. 12 The relationship of aging time to the numbers of micro-organisms on the forward rib area of beef carcasses stored under industrial conditions. Pseudomonad count on the B medium of King. See Appendix VI.

the RH maintained in the local packinghouses might have had a pronounced effect on inhibiting surface microbial development. Rey et al. (1970) did not report the humidity level used in their experiments and since the temperature used in that work (16°) was close to that used in our experiments (15°), it was considered that our high RH (95%) might have been the factor that contributed to the rapid microbial development on meat stored in our laboratory experiments. It seemed therefore worthwhile to investigate the effect of different relative humidities at various temperatures on microbial development on meat stored in the laboratory.

After the dilutions were prepared and plated for the carcass swabs a salmonella enrichment procedure was done on each one as described in the methods section. No salmonellae were recovered from the 90 swabs examined.

As meat from carcasses that were 24 hr. old did not spoil as rapidly as freshly butchered beef, carcass meat that had been stored for approximately 24 hr. was used throughout the remainder of the study. Samples were prepared in a manner similar to the previous experiments and stored for 4, 5 and 6 days at 95, 40 and less than 1% RH. Figs. 13 and 14 do not suggest that meat stored at less than 1% RH produce counts markedly lower than meat stored at the higher relative humidities. In a repeat of the experiment (Figs. 15 and 16), meat stored at 95% RH spoiled as rapidly as it had in the previous trial. However, the meat at the lower humidity levels did not reach as high a microbial count as observed in the first experiment. The extent of bacterial growth at 40 and 1% was similar after 5 and 6 days respectively. Samples stored at the low levels did appear to be dried and some discolouration resulted. Aging at such

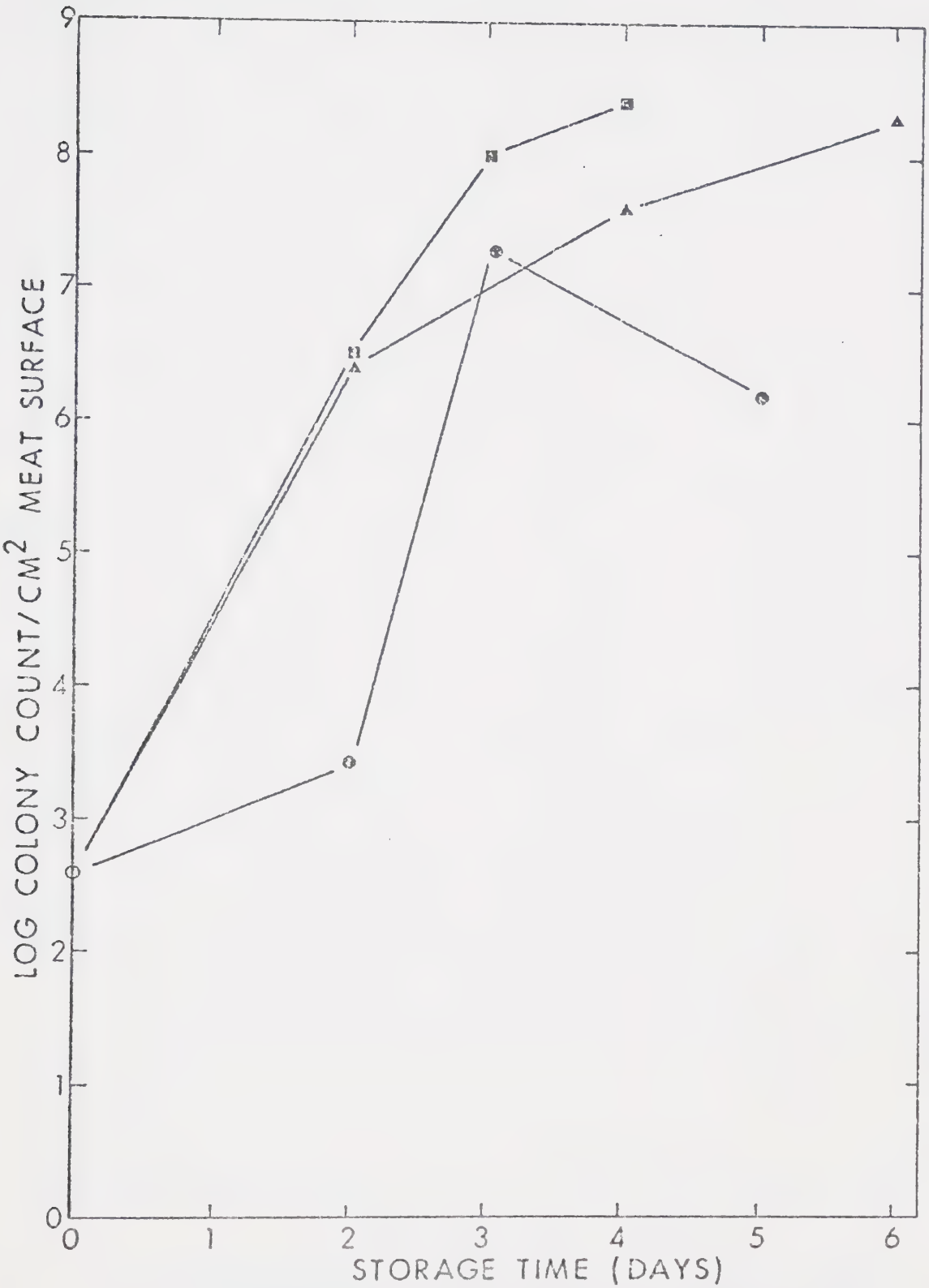

Fig. 13 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 10° and various relative humidities. TPC: O day 0 control; ■ 95%; ● 40%; ▲ less than 1%. See Appendix VII A.

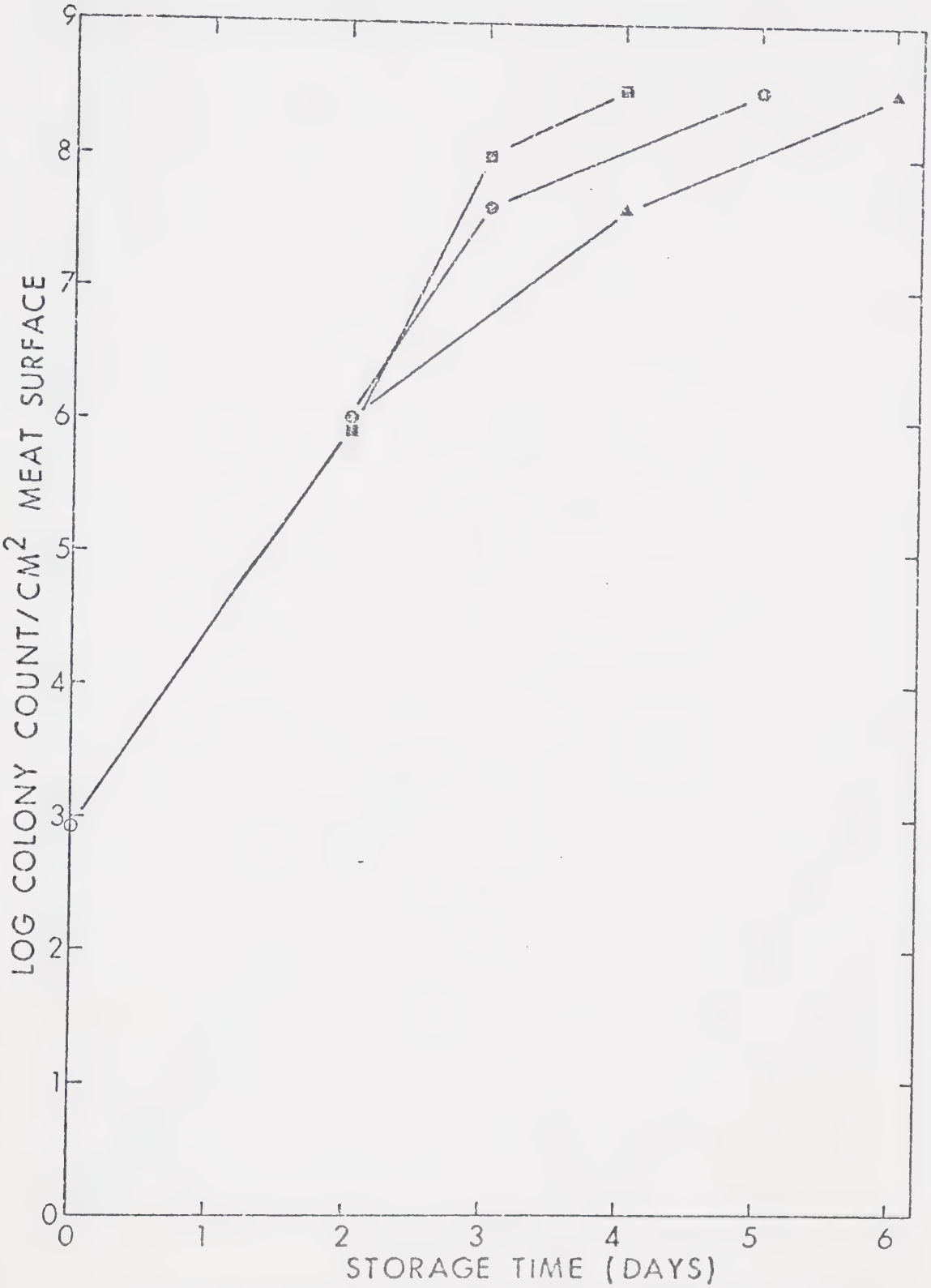

Fig. 14 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 10° and various relative humidities. PC: O day 0 control; ▣ 95%; ⊖ 40%; ▲ less than 1%. See appendix VII A.

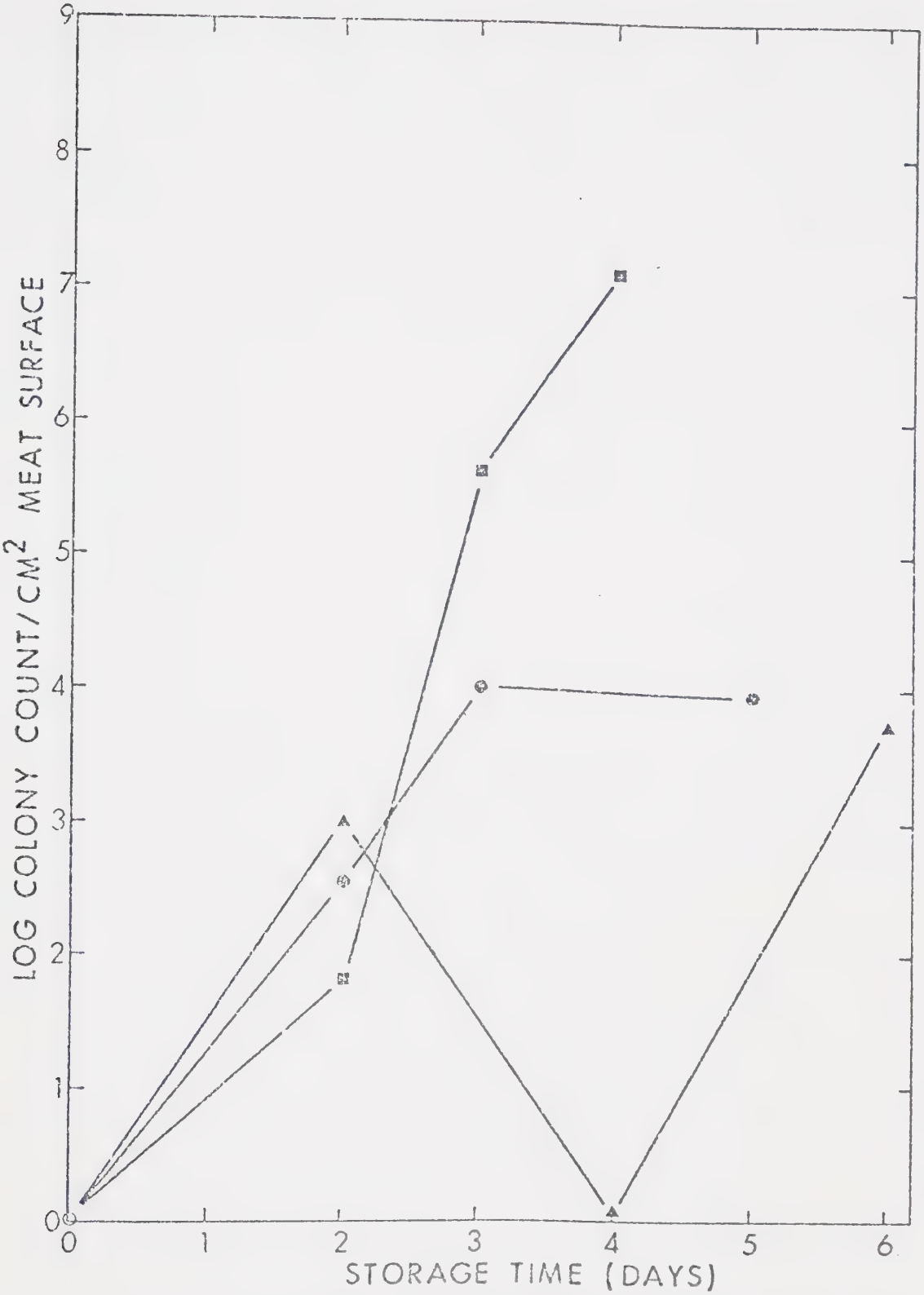

Fig. 15 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 10° and various relative humidities. TPC: O day 0 control; ■ 95%; ● 40%; ▲ less than 1%. See Appendix VII B.

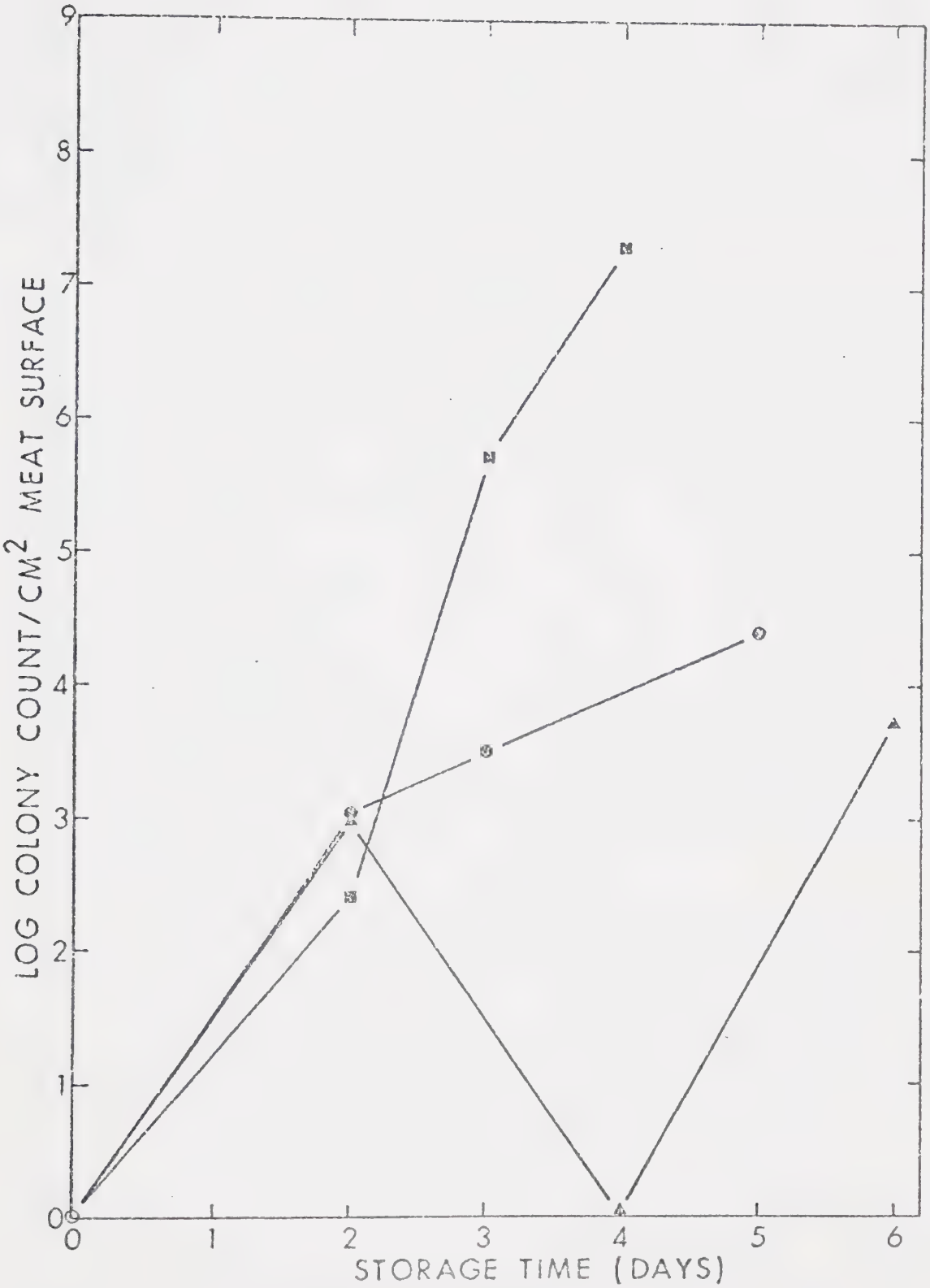

Fig. 16 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 10° and various relative humidities. PC: O day 0 control; ■ 95%; ● 40%; ▲ less than 1%. See Appendix VII B.

low RH would probably result in uneconomical weight loss although no attempt was made to determine the weight loss under the various aging conditions. This suggests that there might be a serious disadvantage in aging meat at extremely low relative humidities.

The following set of experiments were done to study the effect of RH in a more practical range (75-95%) and also to observe whether these had the same effect at various temperatures (2^{o}-12.5^{o}). Figs. 17-20 summarize the TPC and PC on flank steak stored at 75, 85 and 95% RH and at 12.5^{o}. In both pairs of graphs (17-18 and 19-20) the counts exceeded $10^{6}/cm^{2}$ within four days of storage. Counts at the highest RH (95%) were greater in most cases but the differences were not large. There was little difference in counts/cm^{2} on samples stored at 75 and 85%. These These results showed that rapid growth resulted when beef was stored at 12.5^{o} and this temperature is probably too high for beef aging. Unless some method is used to reduce the initial contamination or to prevent growth, aging at 12.5^{o} is not practical.

A similar experiment was done except that the storage temperature was 10^{o}. In the first trial at this temperature (Figs. 21, 22 and 23) counts were again highest when the meat was aged at 95% RH. Results at 75 and 85% were also similar. Pseudomonad counts were initially low but after four days storage they were higher than the TPC at 37^{o}. In the second experiment (Figs. 24, 25 and 26) the counts on day 0 were higher ($10^{4}/cm^{2}$ as compared to $10^{1}/cm^{2}$ in the first trial at 10^{o}). The growth of surface contamination was rapid on samples at all RH and the $10^{6}/cm^{2}$ level was exceeded after the first two days of storage. In this experiment also, the samples stored at 95% RH, generally had the highest counts.

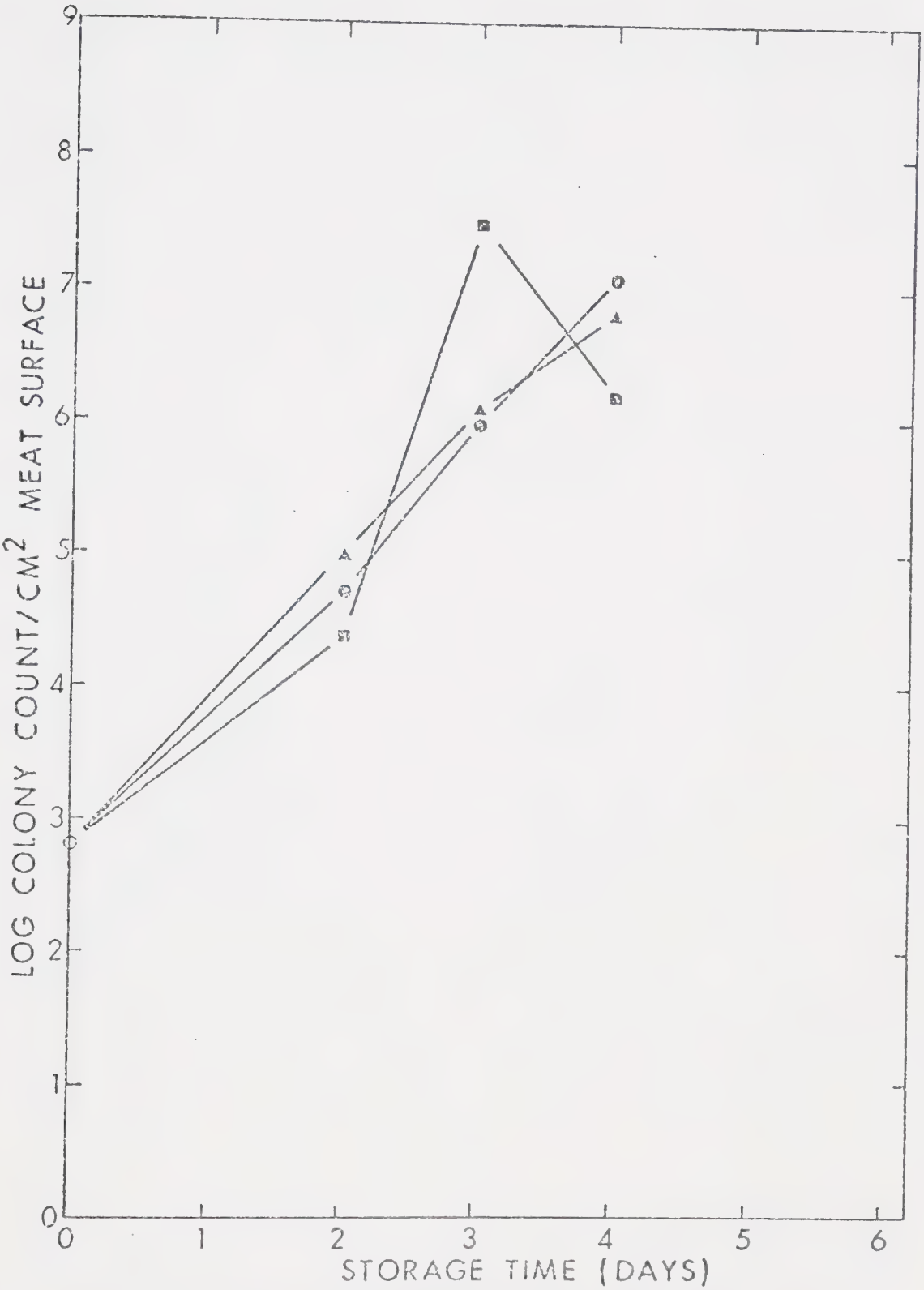

Fig. 17 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 12.5° and various relative humidities. TPC: O day 0 control; ■ 95%; ● 85%; ▲ 75%. See Appendix VIII A.

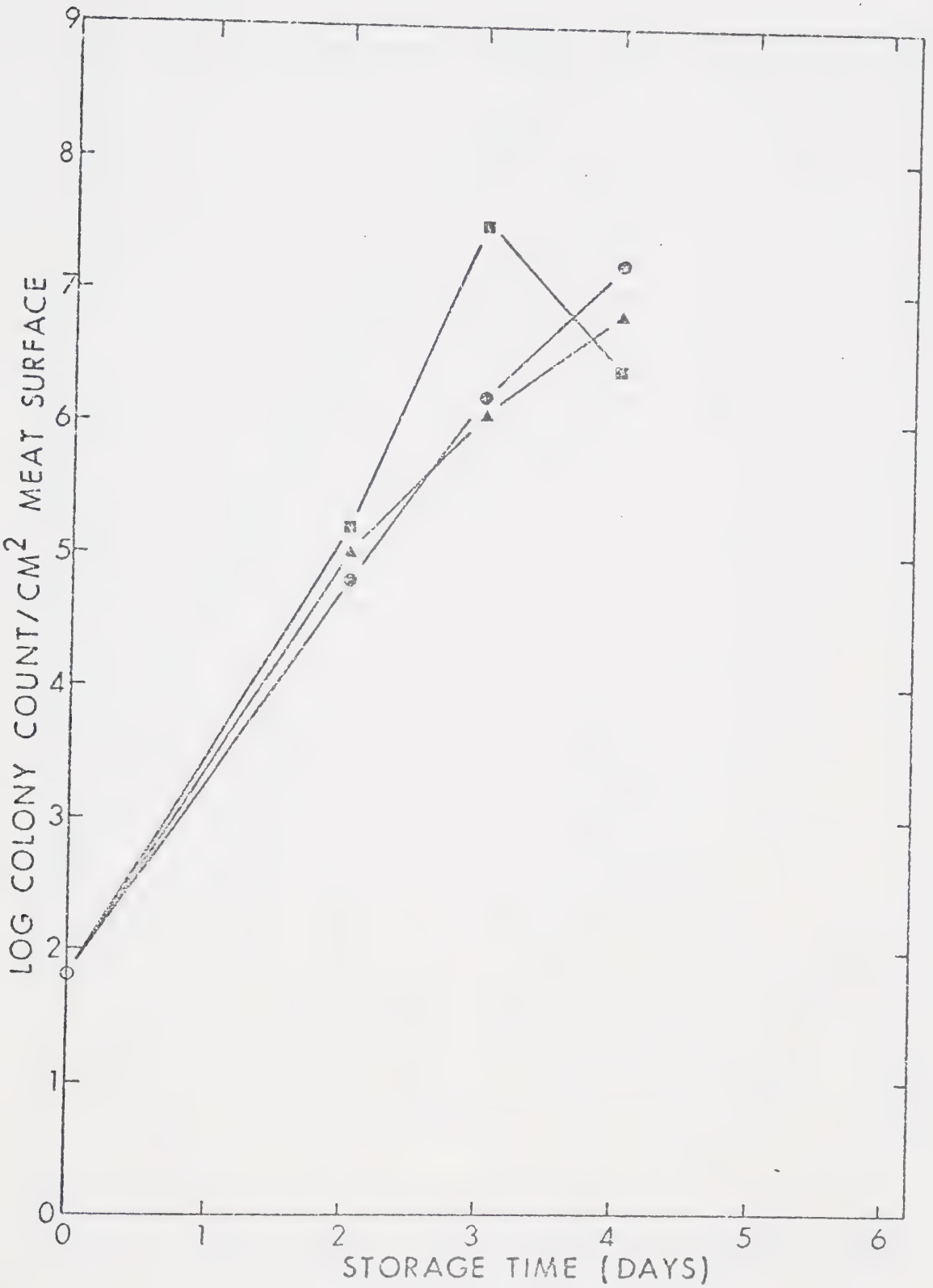

Fig. 18 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 12.5° and various relative humidities. PC: O day 0 control; ■ 95%; ● 85%; ▲ 75%. See Appendix VIII A.

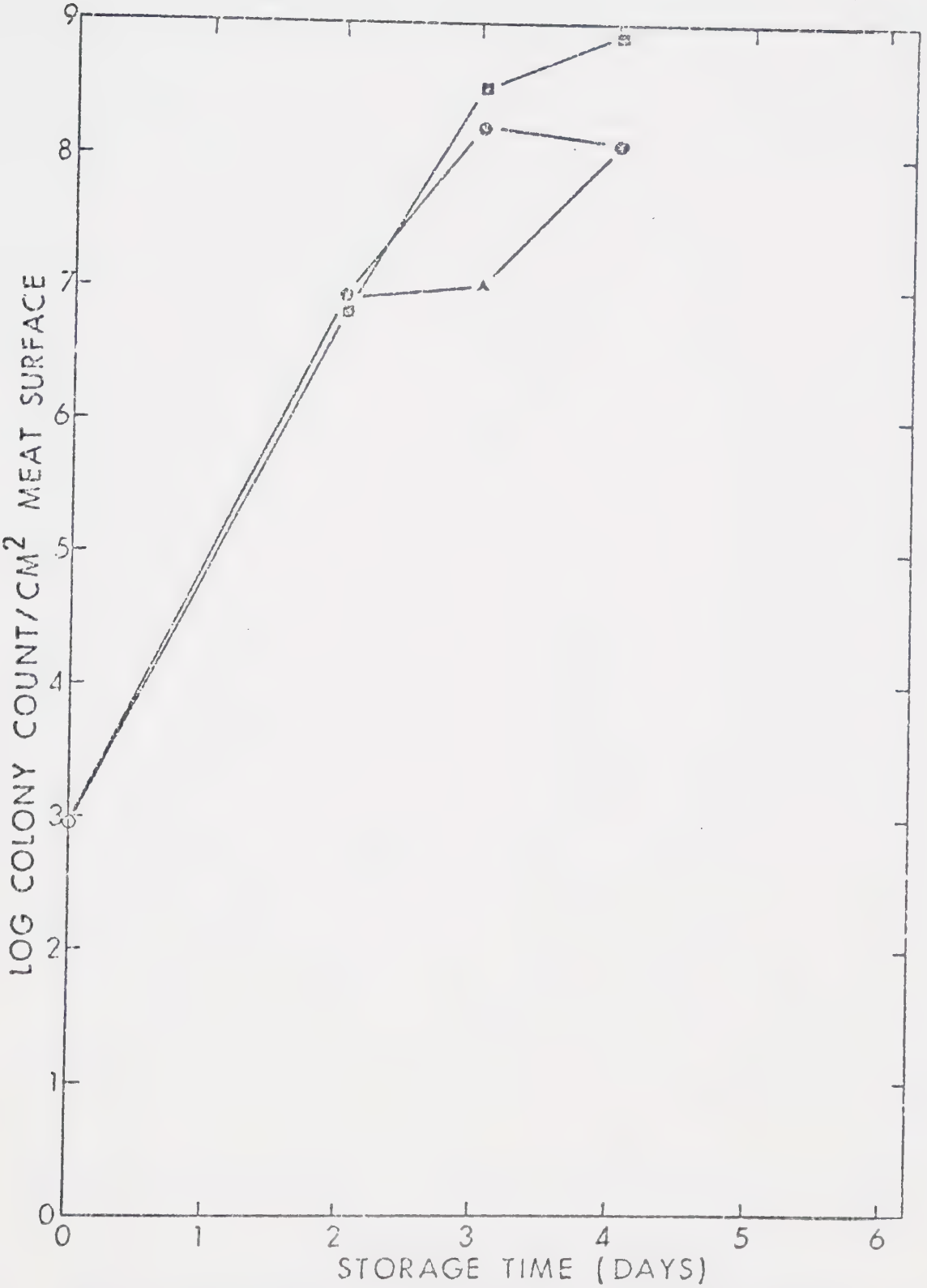

Fig. 19 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 12.5° and various relative humidities. TPC: O day 0 control; ■ 95%; ● 85%; ▲ 75%. See Appendix VIII B.

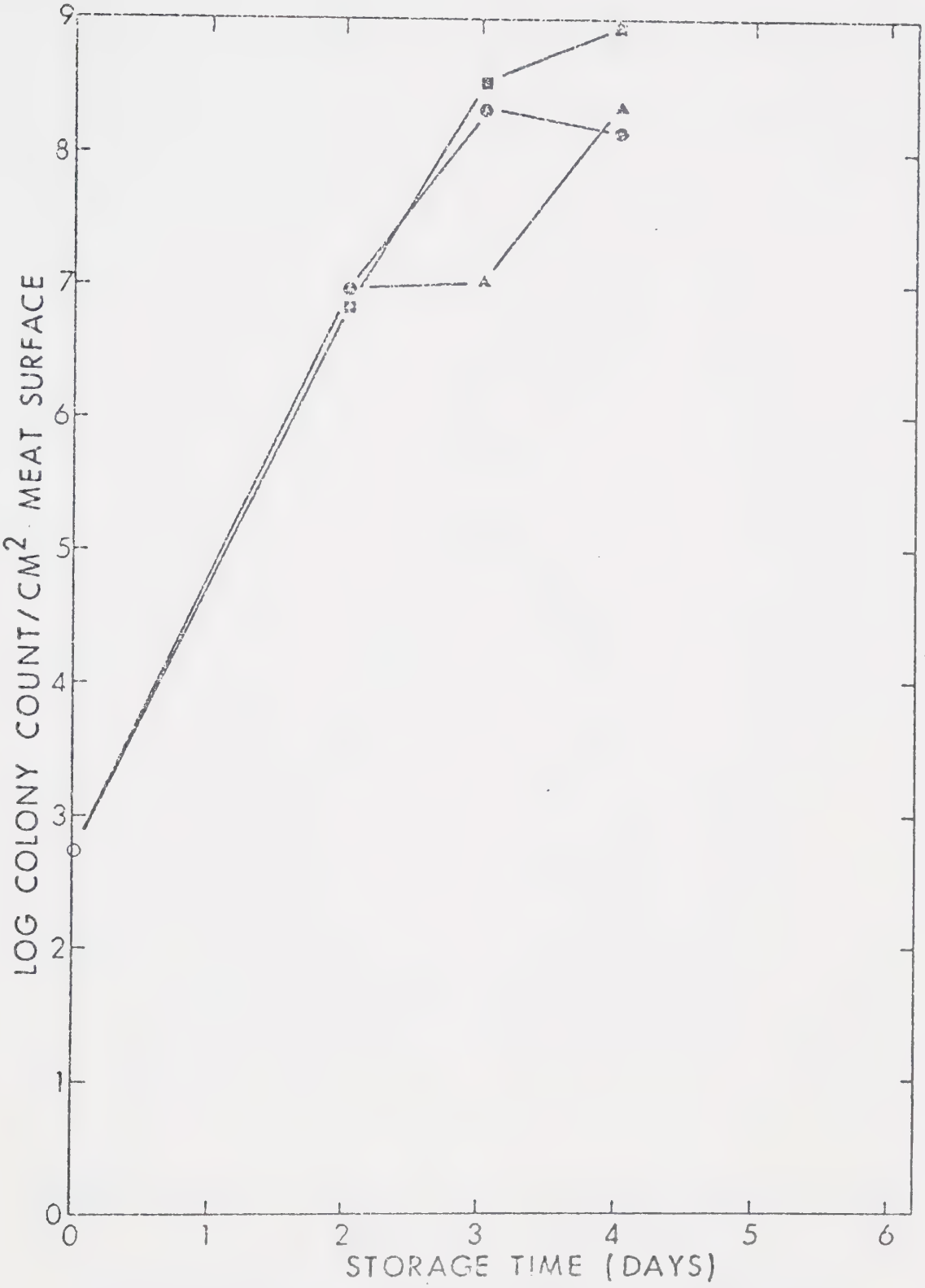

Fig. 20 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 12.5° and various relative humidities. PC: O day 0 control; ■ 95%; ● 85%; ▲ 75%. See Appendix VIII B.

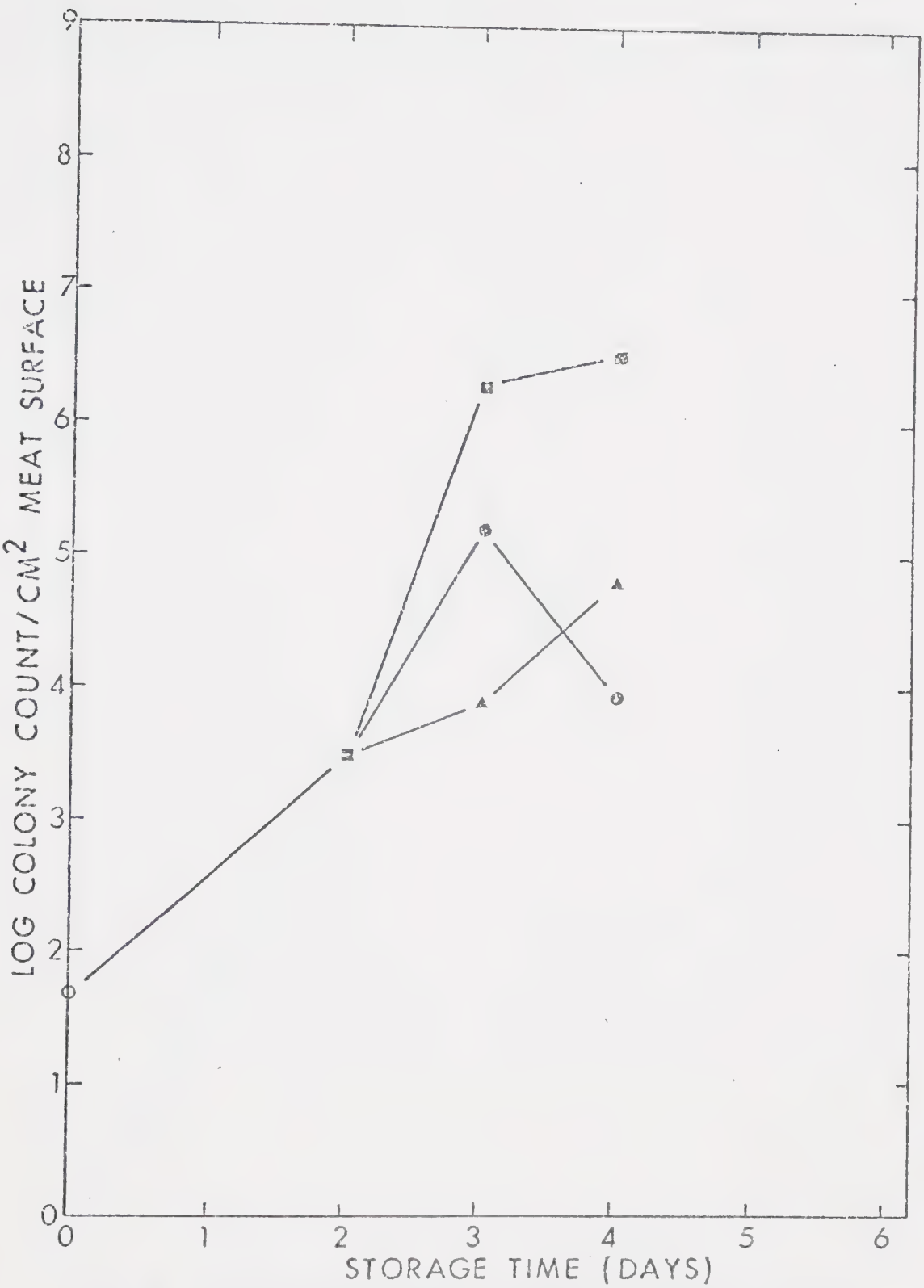

Fig. 21 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 10° and various relative humidities. TPC: O day 0 control; ■ 95%; ●85%; ▲75%. See Appendix IX A.

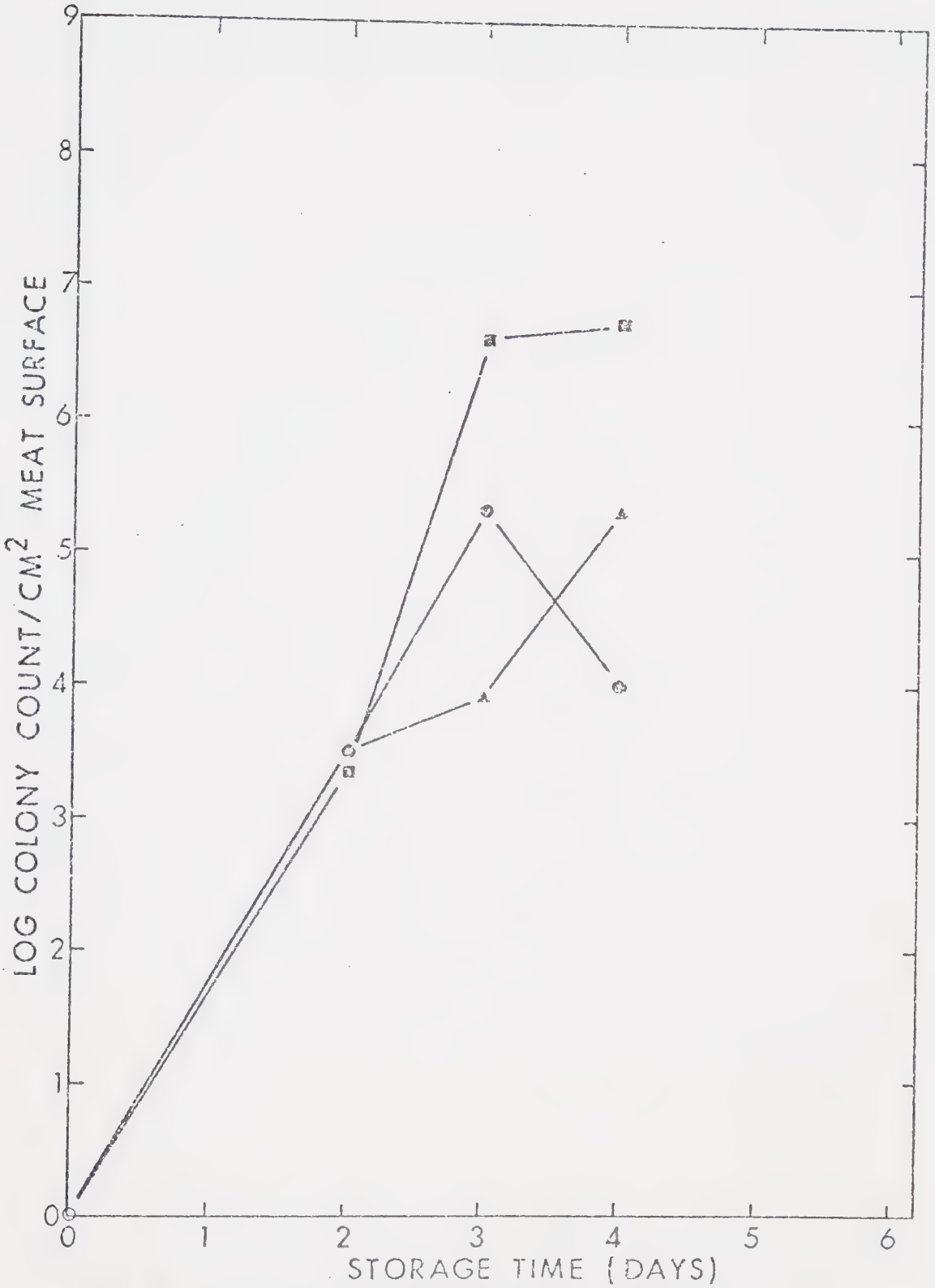

Fig. 22 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 10° and various relative humidities. PC: O day 0 control; ◙ 95%; ● 85%; ▲ 75%. See Appendix IX A.

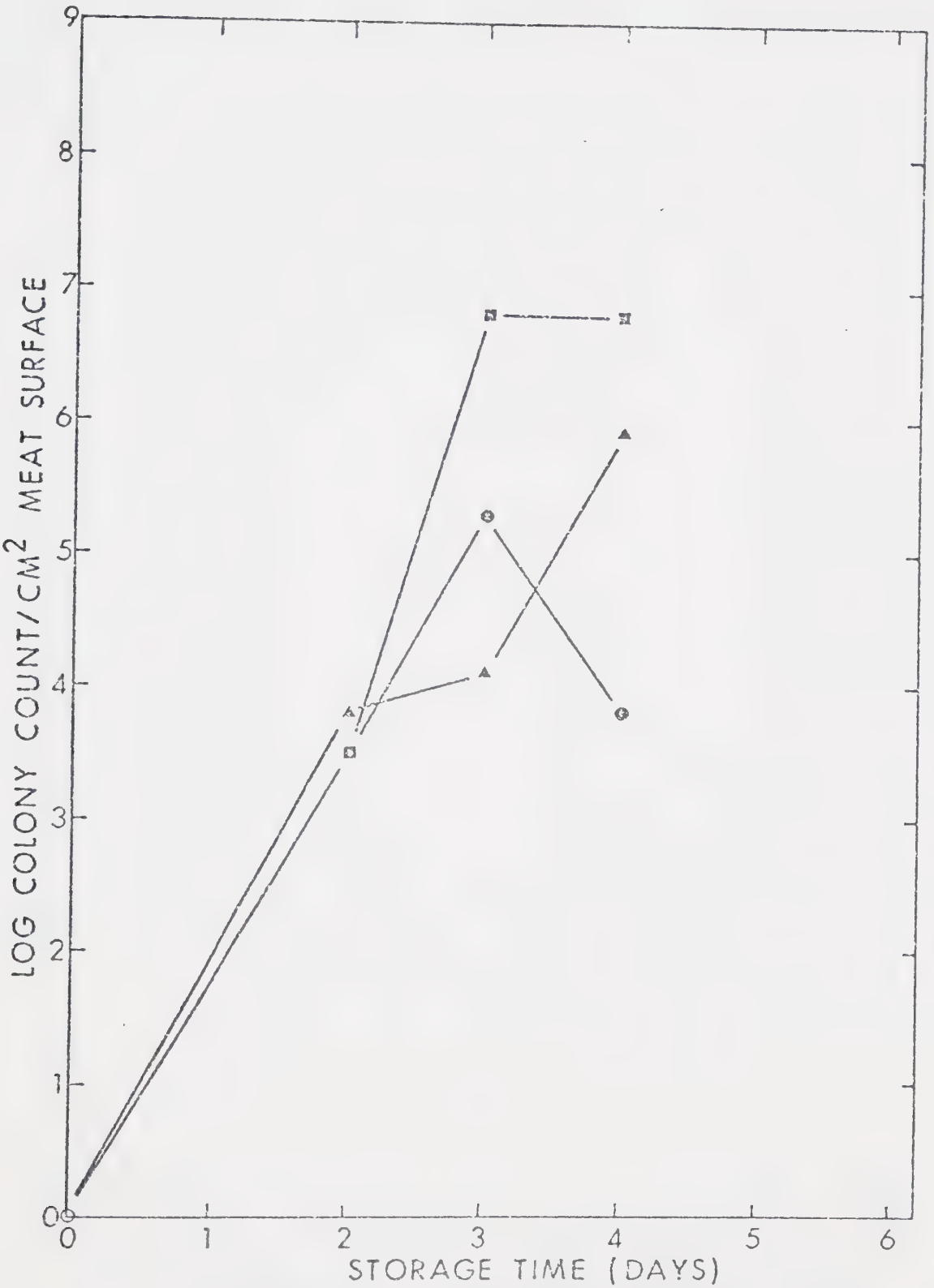

Fig. 23 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 10° and various relative humidities. PC at 10°; O day 0 control; ■ 95%; ● 85%; ▲ 75%. See Appendix IX A.

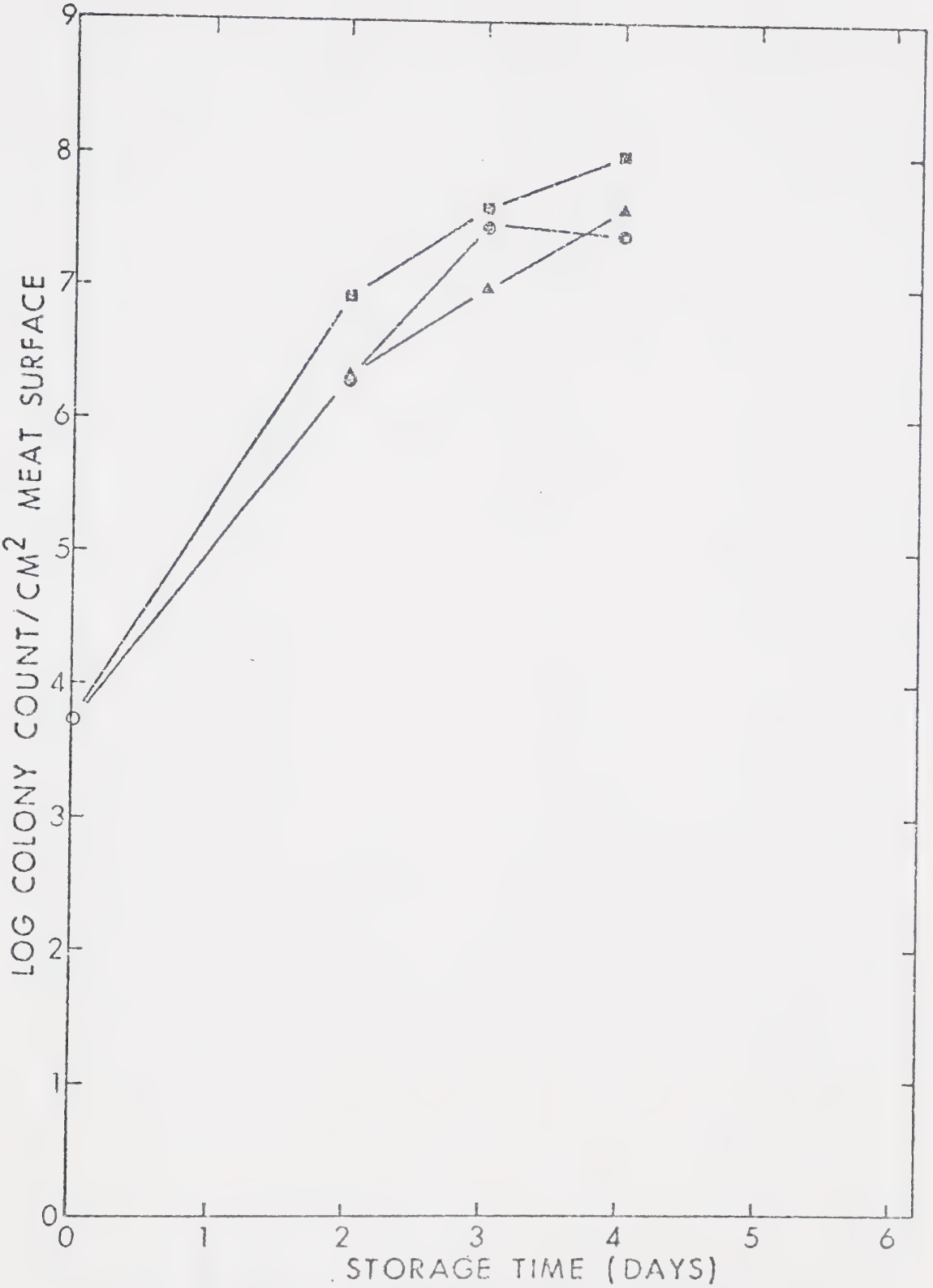

Fig. 24 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 10° and various relative humidities. TPC: O day 0 control; ◘ 95%; ◍ 85%; ▲75%. See Appendix IX B.

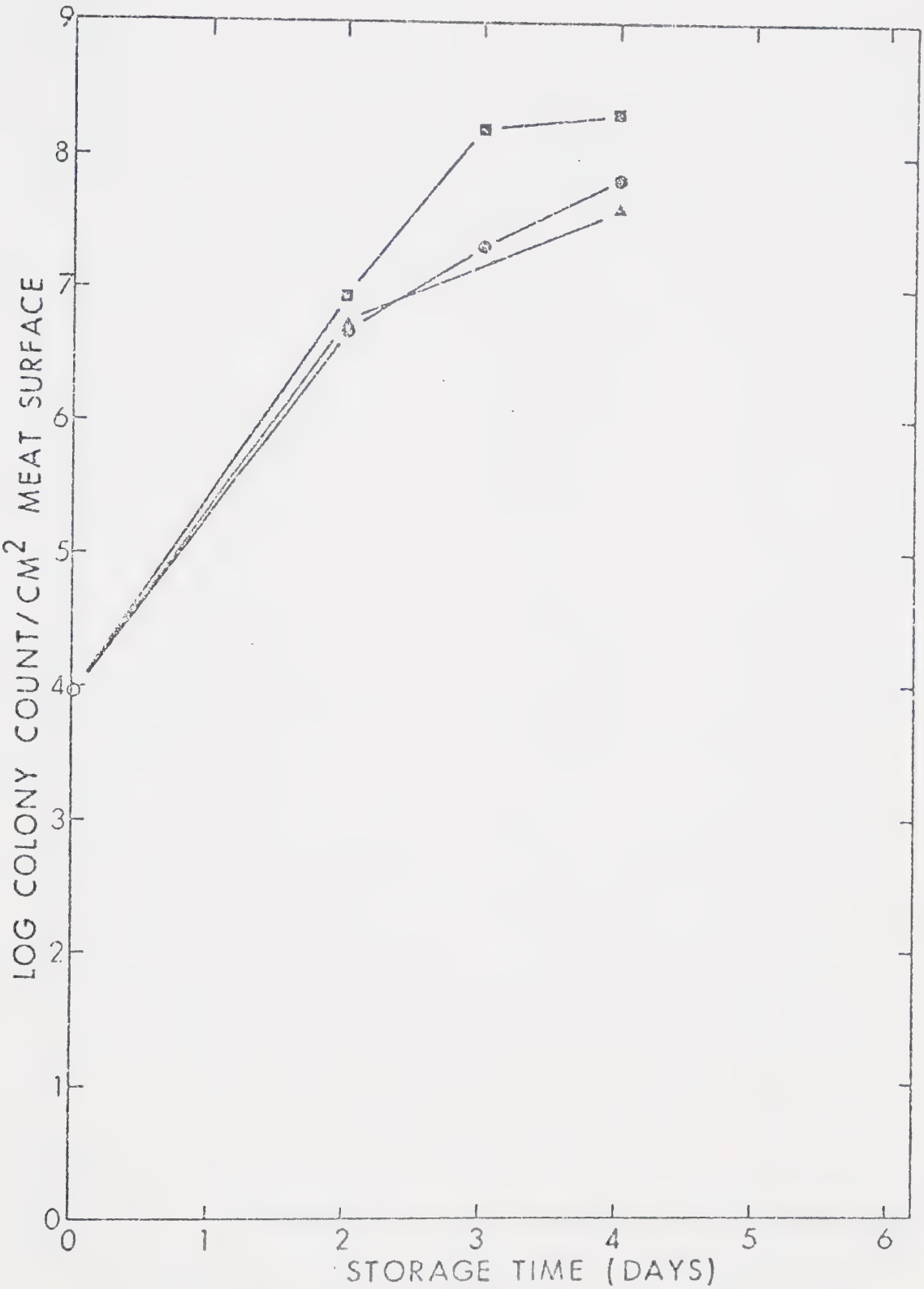

Fig. 25 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 10° and various relative humidities. PC: ○ day 0 control; ■ 95%; ● 85%; ▲ 75%. See Appendix IX B.

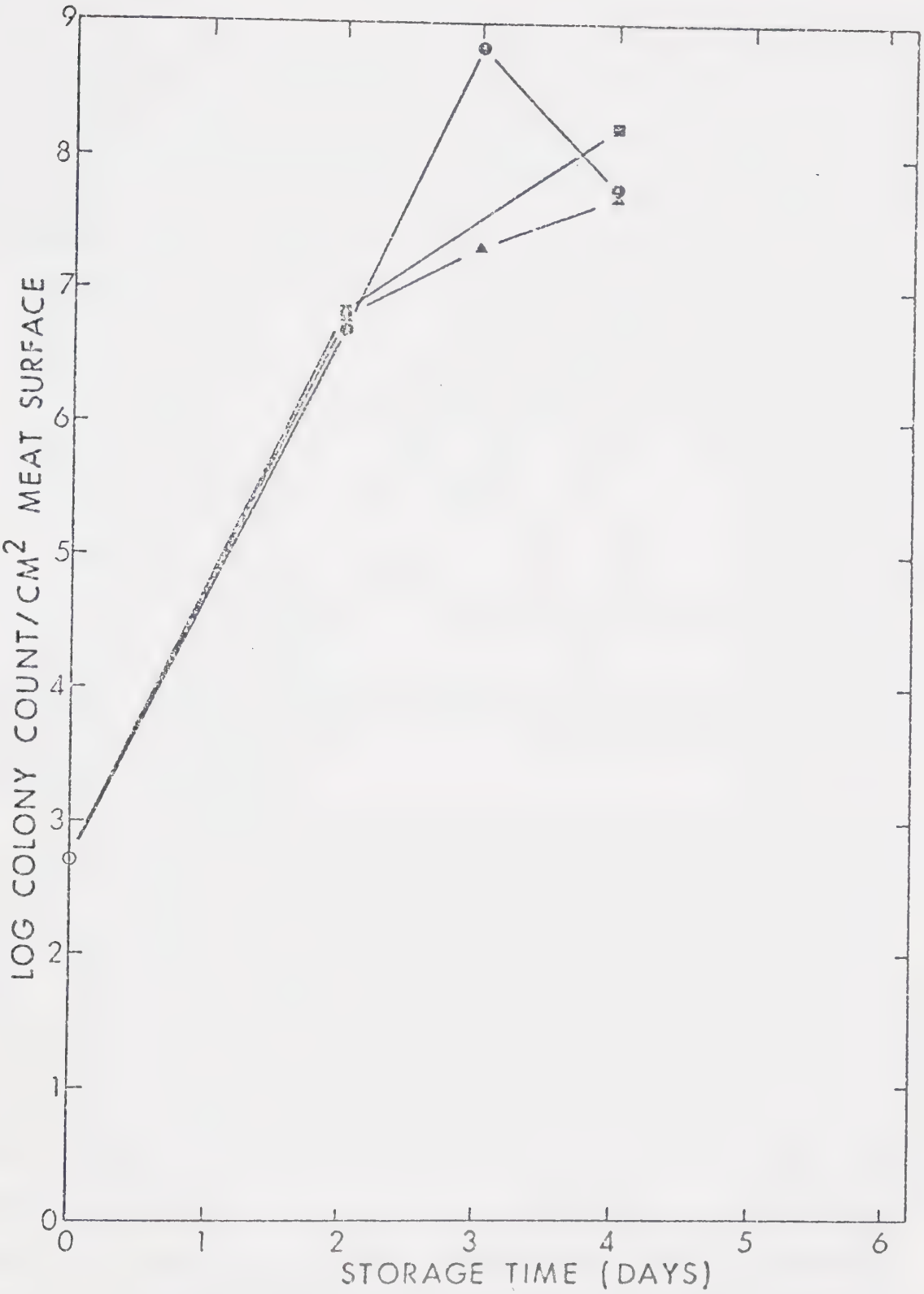

Fig. 26 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 10° and various relative humidities. PC at 10° : O day 0 control; ■ 95%; ● 85%; ▲ 75%. See Appendix IX B.

The differences in counts between the samples were again not great. The counts after four days aging were also higher than the final counts in the first experiment. These results suggest that the initial contamination present on the meat surface is important in determining the rate at which spoilage will proceed. As stated by Elliott and Michener (1965) the higher the initial microbial load the more rapidly spoilage results.

Figs. 27 and 28 are the TPC and the PC of samples stored at 75, 85 and 95% RH and at 4.4^{o} for eight days. In this experiment counts at all three RH were similar. The counts at 95% were not higher than those at 75 and 85% as had been experienced in the trials at high temperatures ie. 10^{o} and 12.5^{o}. After eight days of aging all the counts exceeded $10^{6}/cm^{2}$. The day 0 counts on these samples were moderately high so this might have partially accounted for the high bacterial populations at the end of the aging period.

The variation in the initial levels of surface contamination created problems in this work. There was no means of predicting what amount of contamination would be present when the meat was sampled and thus to estimate how rapidly the meat might be expected to spoil. Even at the lowest temperature of laboratory aging (2^{o}), the meat could spoil rapidly if the right type of micro-organisms were present. In Figs. 29 and 30 the TPC and PC for flank steak stored at 2^{o} and 75, 85 and 95% RH are shown. At this temperature, as at 4.4^{o}, there was little difference between the counts at the various relative humidities. Spoilage was rapid and the counts approached $10^{6}/cm^{2}$ within the first four days of aging. After six days the counts had exceeded $10^{6}/cm^{2}$ and on the eighth day colonies were visible growing on the surface of the meat as illustrated

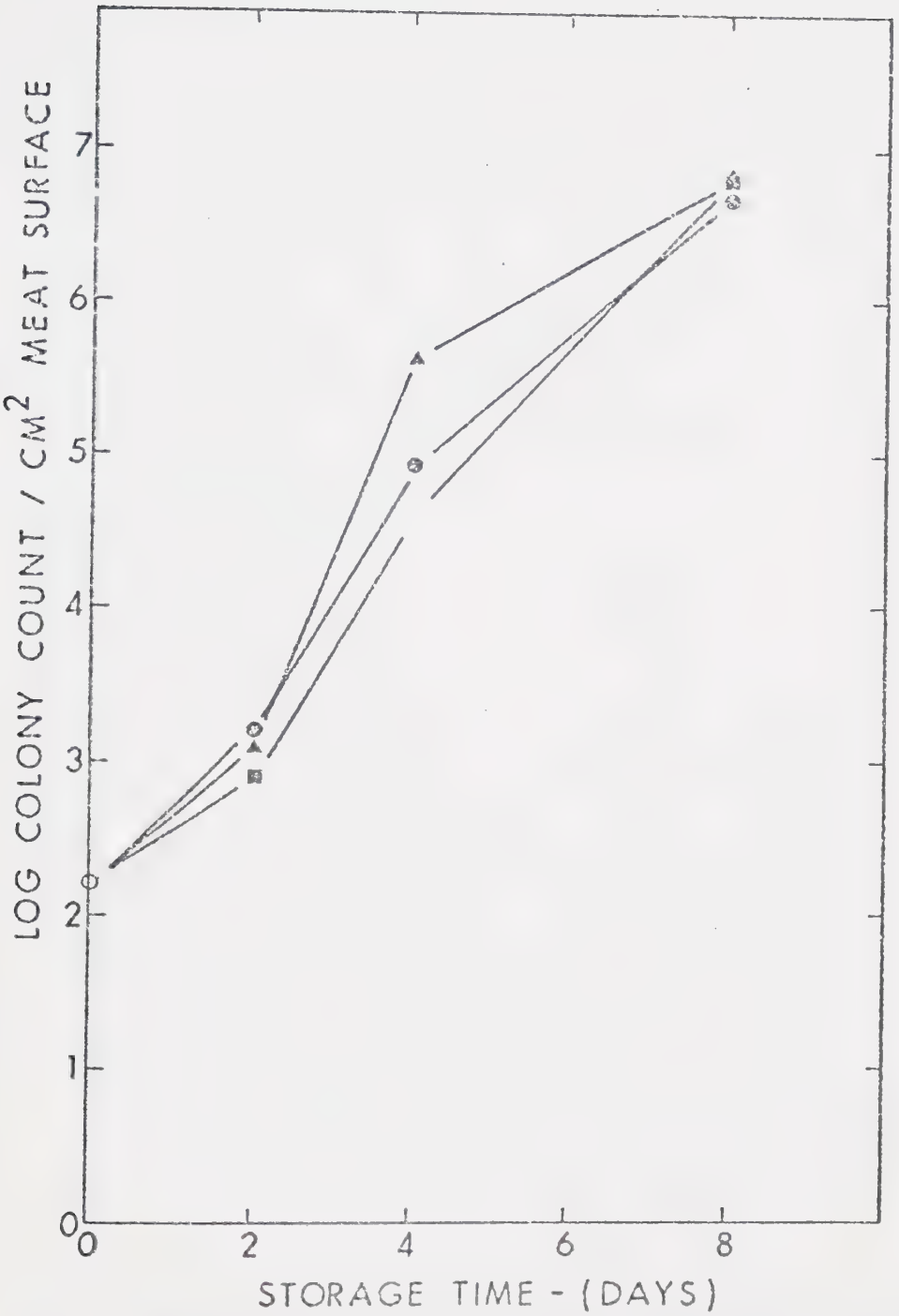

Fig. 27 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 4.4° and various relative humidities. TPC: O day 0 control; ■ 95%; ● 85%; ▲ 75%. See Appendix X A.

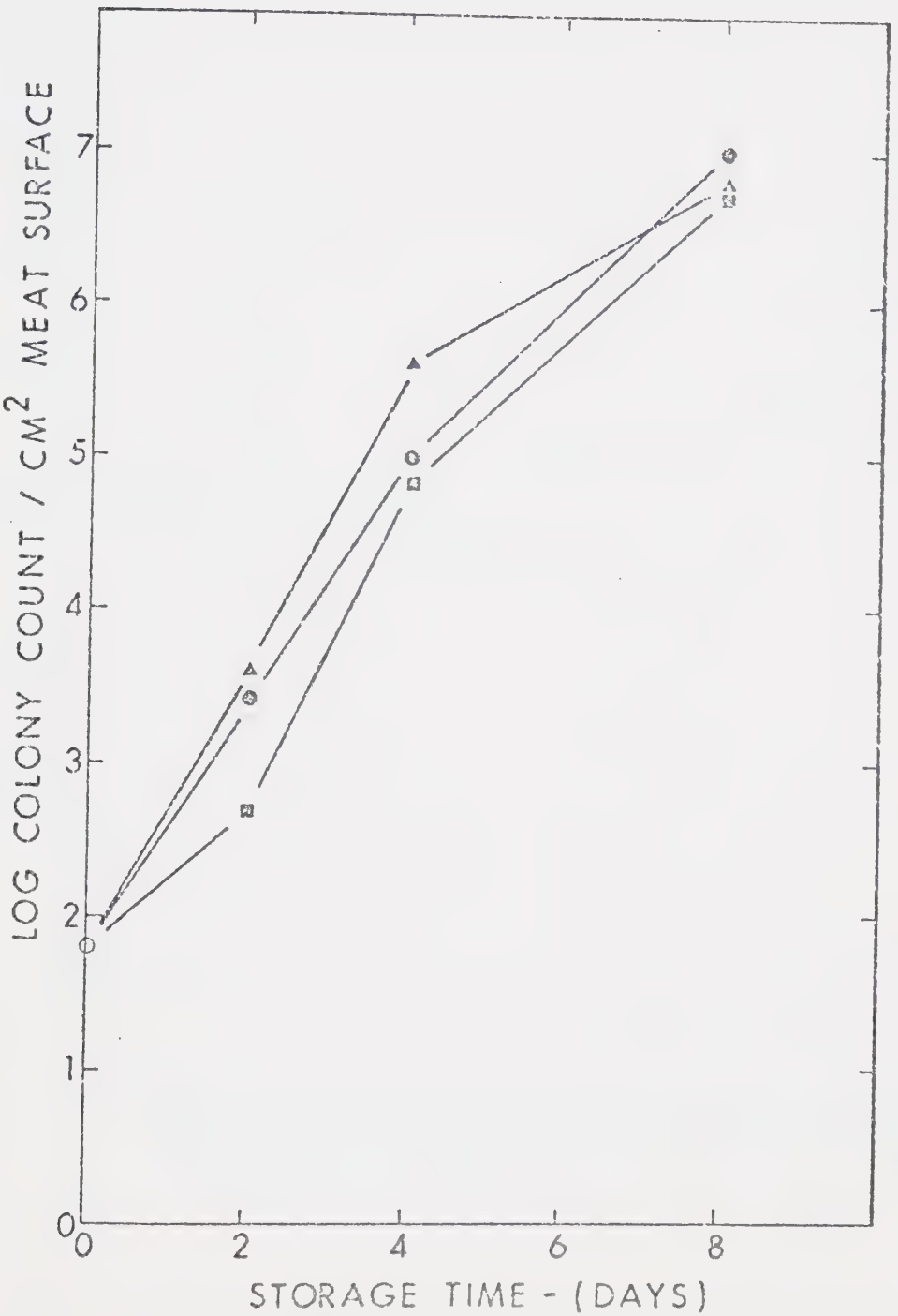

Fig. 28 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 4.4° and various relative humidities. PC: O day 0 control; ◙ 95%; ● 85%; ▲ 75%. See Appendix X A.

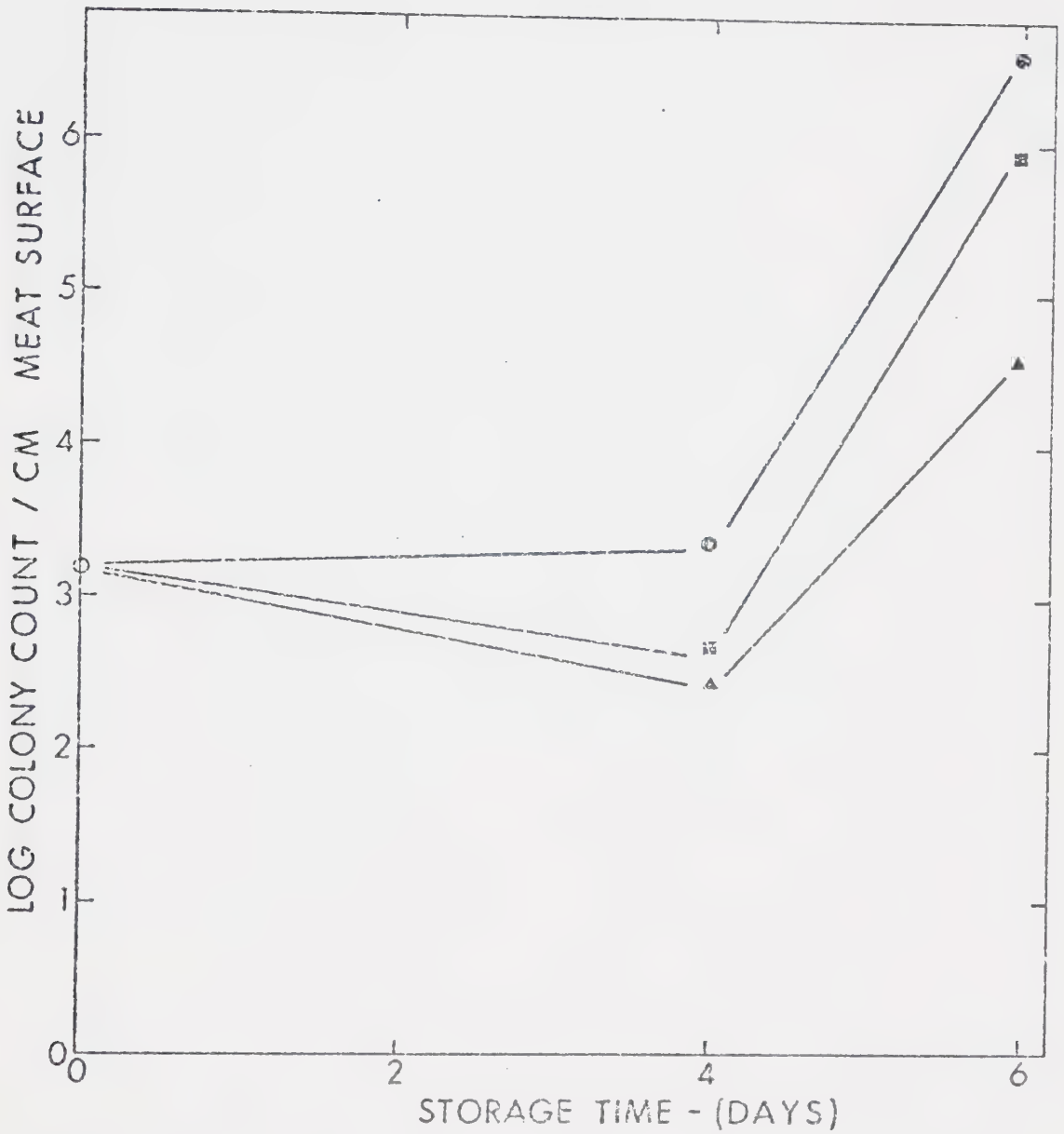

Fig. 29 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 2° and various relative humidities. TPC: O day 0 control; ■ 95%; ● 85%; ▲ 75%. See Appendix X B.

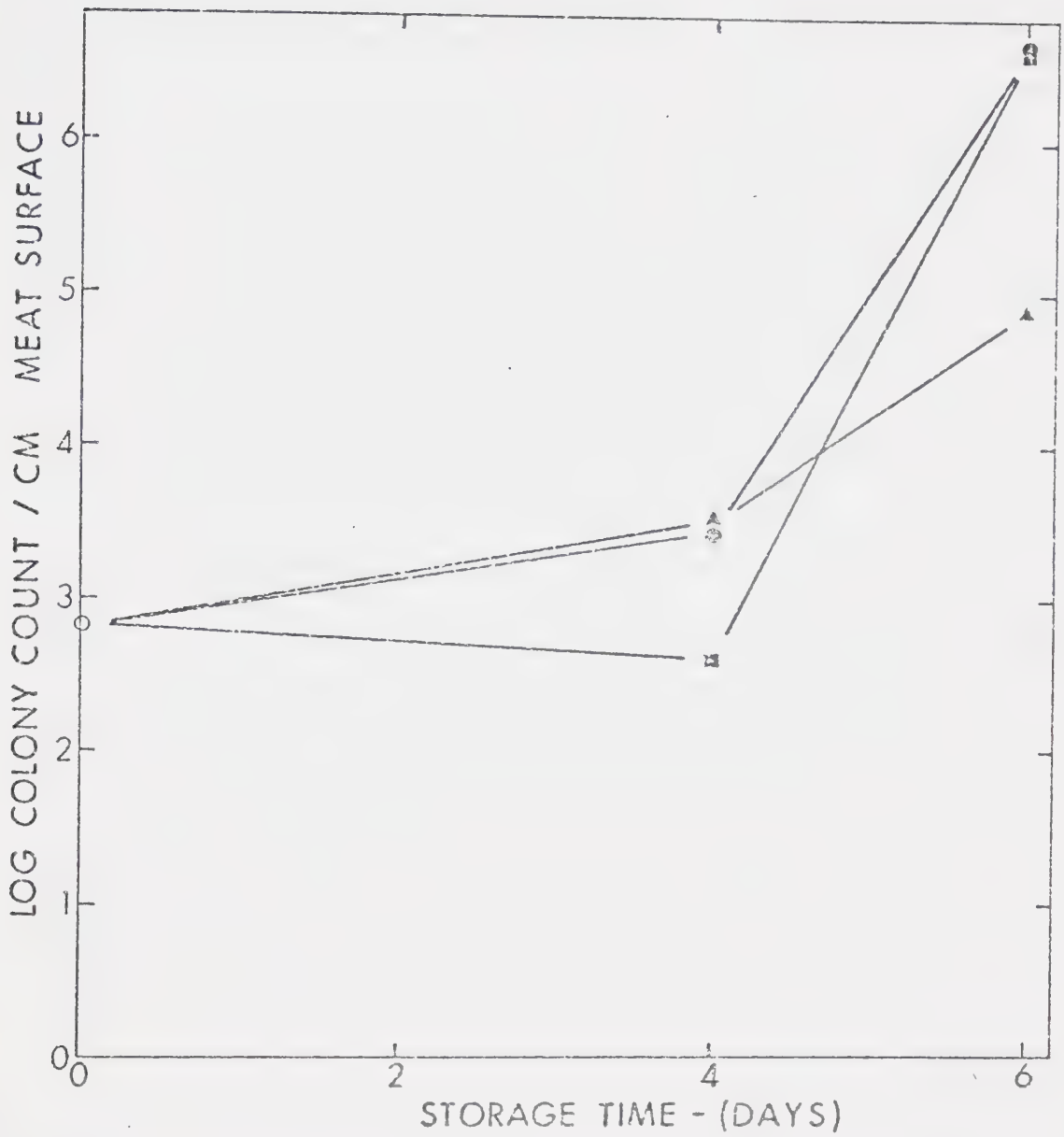

Fig. 30 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 2° and various relative humidities. PC: O day 0 control; ■ 95%; ● 85%; ▲ 75%. See Appendix X B.

in Fig. 31. The results of this experiment again demonstrated that the numbers and optimum growth temperatures of the initial contamination on a meat surface are important in determining the rate of spoilage. These samples were stored at a low temperature (normal for a packinghouse aging room) and spoilage should have been minimal. However the micro-organisms that were present had the ability to grow at this low temperature and thus were able to form colonies on the meat surface within eight days.

The experiments involving the aging of meat at various relative humidities showed that there was no advantage in aging at very low levels such as 40 and 1%. Furthermore aging in this humidity region would probably cause excess weight loss and did not seem to inhibit microbial growth markedly. Again experiments at 75, 85 and 95% RH indicated that aging at 95% RH could lead to more rapid spoilage and that there was little or no difference in aging at 75 or 85% RH with regards to microbial development on meat surfaces. As weight loss would be less at 85% RH, this was accordingly chosen as the condition to be used in subsequent experiments.

The extent of surface contamination is important in determining the rate of spoilage so the final experiments involved attempts to remove or retard the growth of the naturally occuring contaminates with chlorine or hot water sprays. Chlorine was used at levels of 3 (residual chlorine in tap water), 20 and 500 ppm. The concentration of residual chlorine was higher than might be expected. However, these experiments were done in the spring when chlorine levels are increased in the water supply to compensate for the spring run-off. The residual chlorine levels are usually higher during that period. Samples were stored after treatment

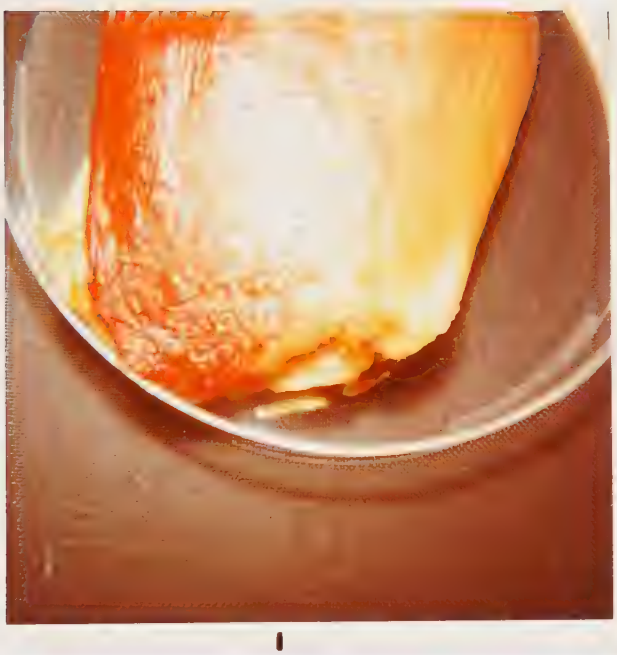

Fig 31 Microbial colonies on the surface of flank steak stored at 2° and 85% relative humidity for 8 days.

for four days at 12.5°. The high storage temperature was used because the effect of the germicidal treatments might be more pronounced. As shown in Figs. 32 and 33 there was only a small reduction in the counts after spraying on day 0. Spoilage was rapid and the $10^6/cm^2$ level was exceeded in all samples after four days. Chlorine at 20 ppm did not prevent growth much more than spraying with tap water containing 3 ppm chlorine. Counts on samples with 500 ppm chlorine sprays were lower at most of the sampling times but the differences were not great. The results of the second experiment (Figs 34 and 35) showed a different effect. The initial counts were low and the rate of microbial development was also lower. Samples sprayed with 3 and 20 ppm chlorine had counts above $10^6/cm^2$ after four days but these were lower than the counts in the first trial. The increase in the counts on the samples treated with 500 ppm chlorine was only apparent after three days and leveled off to 10^3 after the fourth day. It was again evident in these experiments that surface contamination can vary widely. The samples examined on day 0 sprayed with water containing 3 ppm chlorine had a higher count than the samples that were not treated. The overall degree of contamination was highest in the first experiment so the results of the second trial were more satisfactory. Dainty (1971) stated that 20 ppm chlorine spraying had little effect in controlling spoilage and this was also evident in our experiments.

Meat was then sprayed with water of different temperatures in an attempt to lower the initial contamination. The water temperatures used in the first experiment were 18°, 53° and 70°. The volume of water applied to each sample was much greater than that used in the chlorine

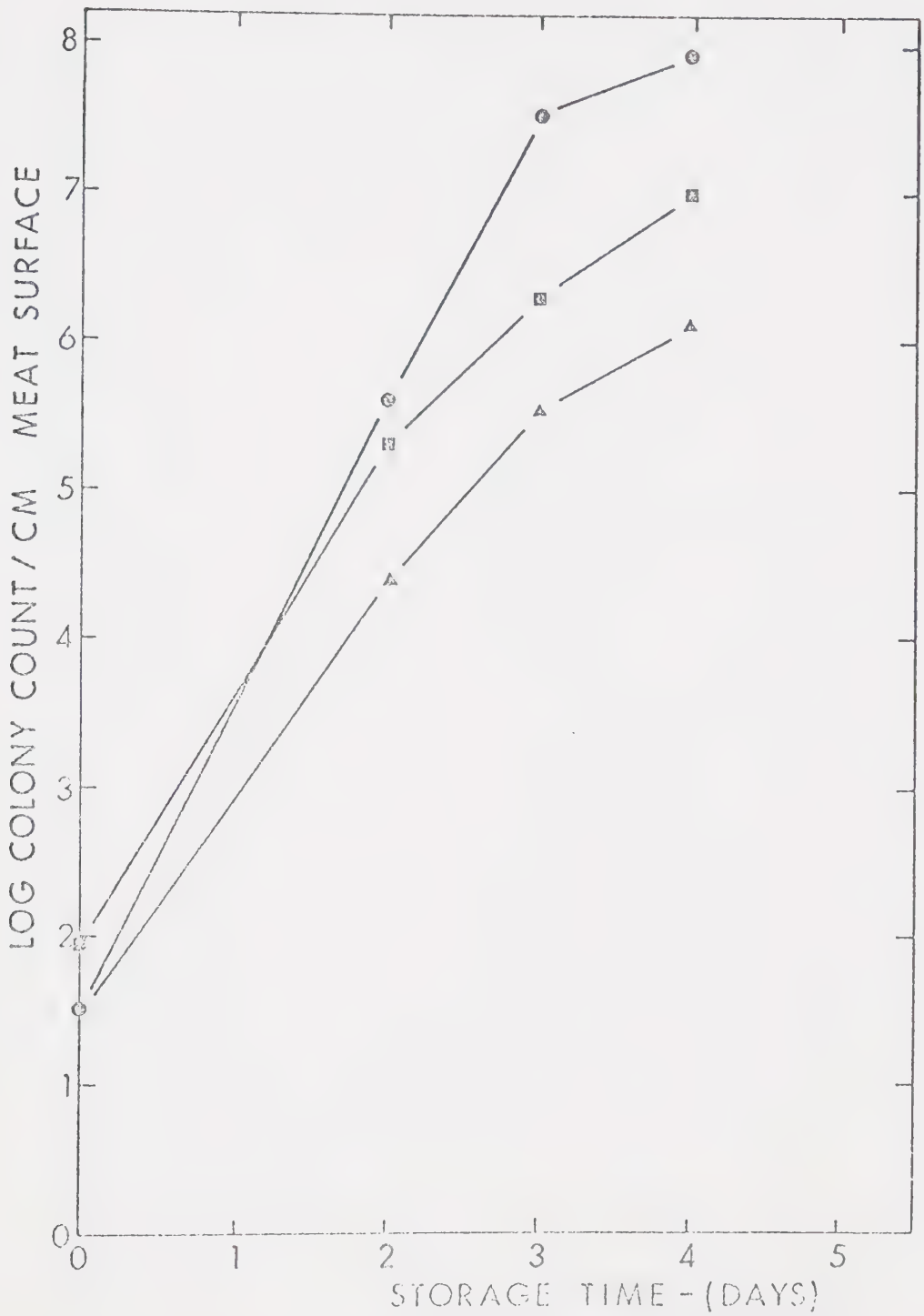

Fig. 32 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 12.5° and 85% RH after treatment with sprays of various chlorine concentrations. TPC: O no treatment; ▣ 3 ppm chlorine; ◉ 20 ppm chlorine; ▲ 500 ppm chlorine. See Appendix XI A.

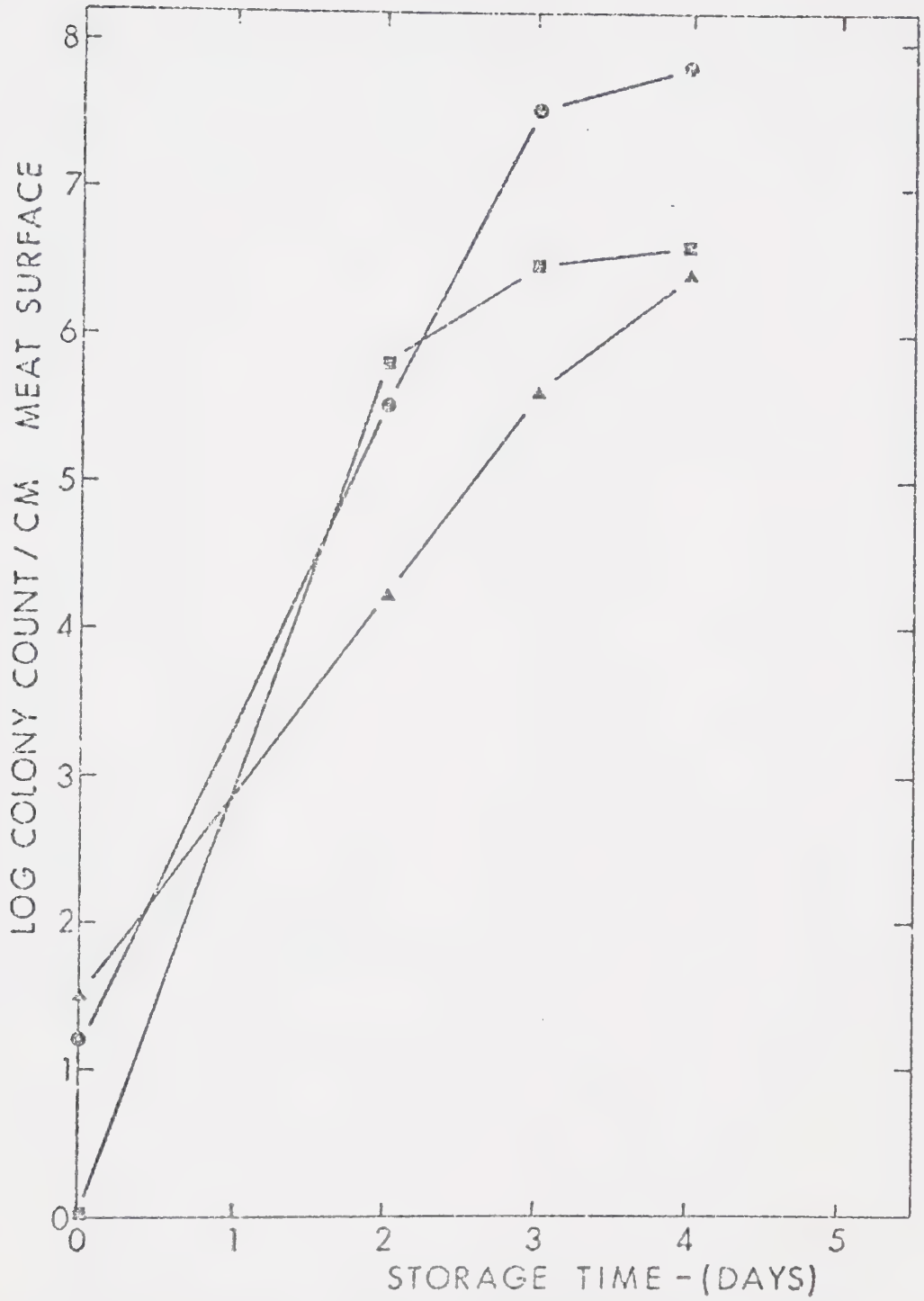

Fig. 33 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 12.5° and 85% RH after treatment with sprays of various chlorine concentrations. PC: ○ no treatment; ■ 3 ppm chlorine; ● 20 ppm chlorine; ▲ 500 ppm chlorine. See Appendix XI A.

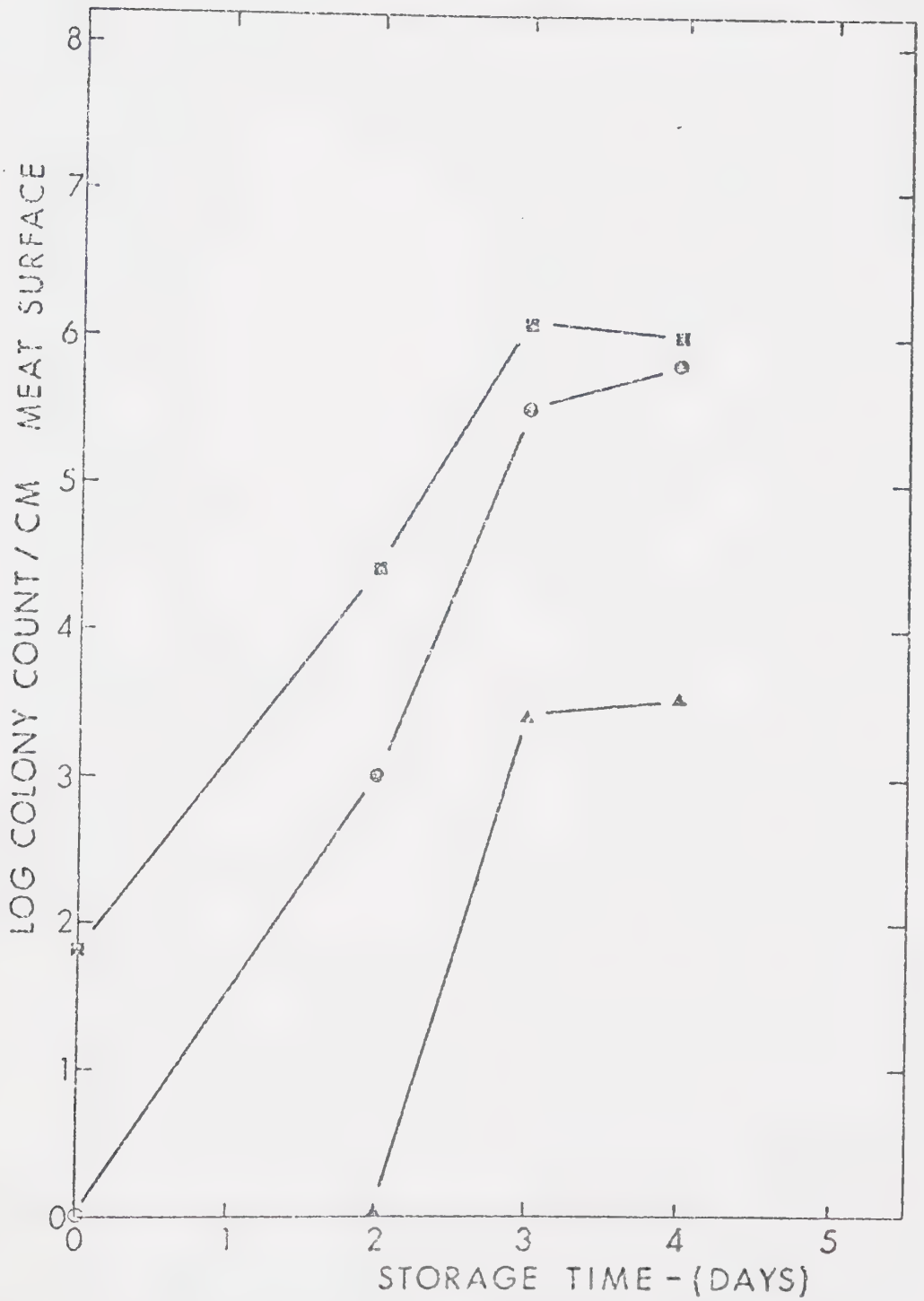

Fig. 34 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 12.5° and 85% RH after treatment with sprays of various chlorine concentrations. TPC: O no treatment; ■ 3 ppm chlorine; ● 20 ppm chlorine; ▲ 500 ppm chlorine. See Appendix XI B.

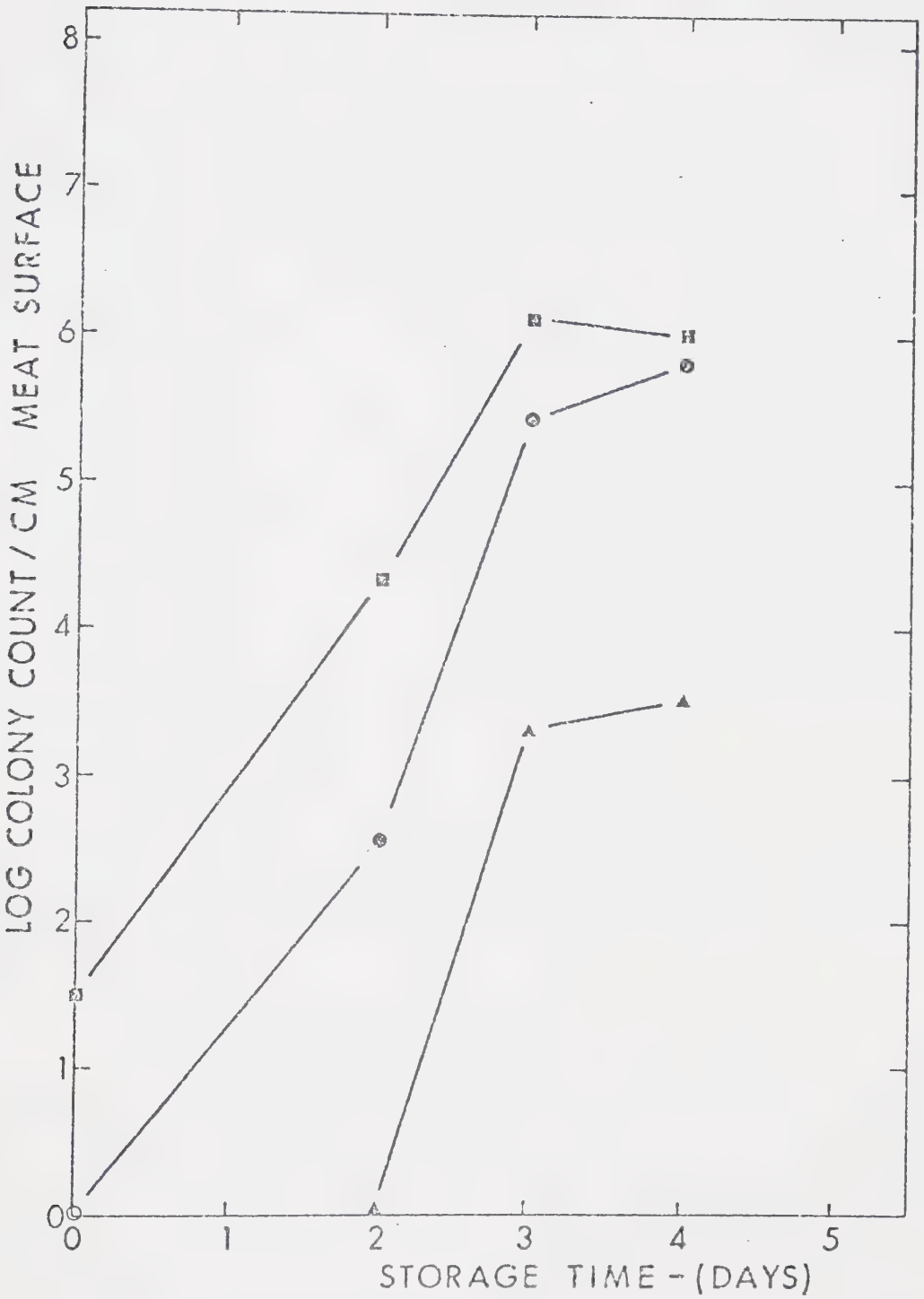

Fig. 35 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 12.5° and 85% RH after treatment with sprays of various chlorine concentrations. PC: O no treatment; ■ 3 ppm chlorine; ◉ 20 ppm chlorine; ▲ 500 ppm chlorine. See Appendix XI B.

spraying. The highest water temperatures available with the equipment used was 70°. The results are shown in Figs. 36, 37, 38 and 39. In Figs. 36 and 37 the final counts after three days storage were highest on the samples sprayed with the water at 70°. These samples had a strong odour and were spoiled after the fourth day of aging. Results of the second trial (Figs. 38 and 39) were similar. The preliminary counts in both experiments were low (less than 33/cm^2) so the microbial development may have been stimulated by the water that was added to the surfaces during spraying. In three of the Figs. (36, 38 and 39) the initial counts on the meat sprayed with water at 70° had higher counts than the samples that were not treated. This was suprising as the 70° water spray probably removed some micro-organisms before the sample was swabbed. Apparently spraying with water at the temperatures used did not inhibit microbial development on the flank steak stored at 12.5°.

Each sample dilution was also plated on EMBA during this work. Coliform contamination could readily be identified on this medium. There were only three incidences of coliform contamination on the meat used in this work.

Salmonella enrichment procedures were done on several samples because Weissman and Carpenter (1968) and Hobbs and Wilson (1959) reported high incidences of salmonella contamination in beef. None of the samples of chuck steak, flank steak or any of the 90 swabs examined were positive for salmonella. During all the experiments, plating on BGA was continued and no positive results were obtained. It seems unlikely therefore that salmonella was prevalent in the packinghouses examined in this study. Although positive samples might have been missed in the BGA plating, the

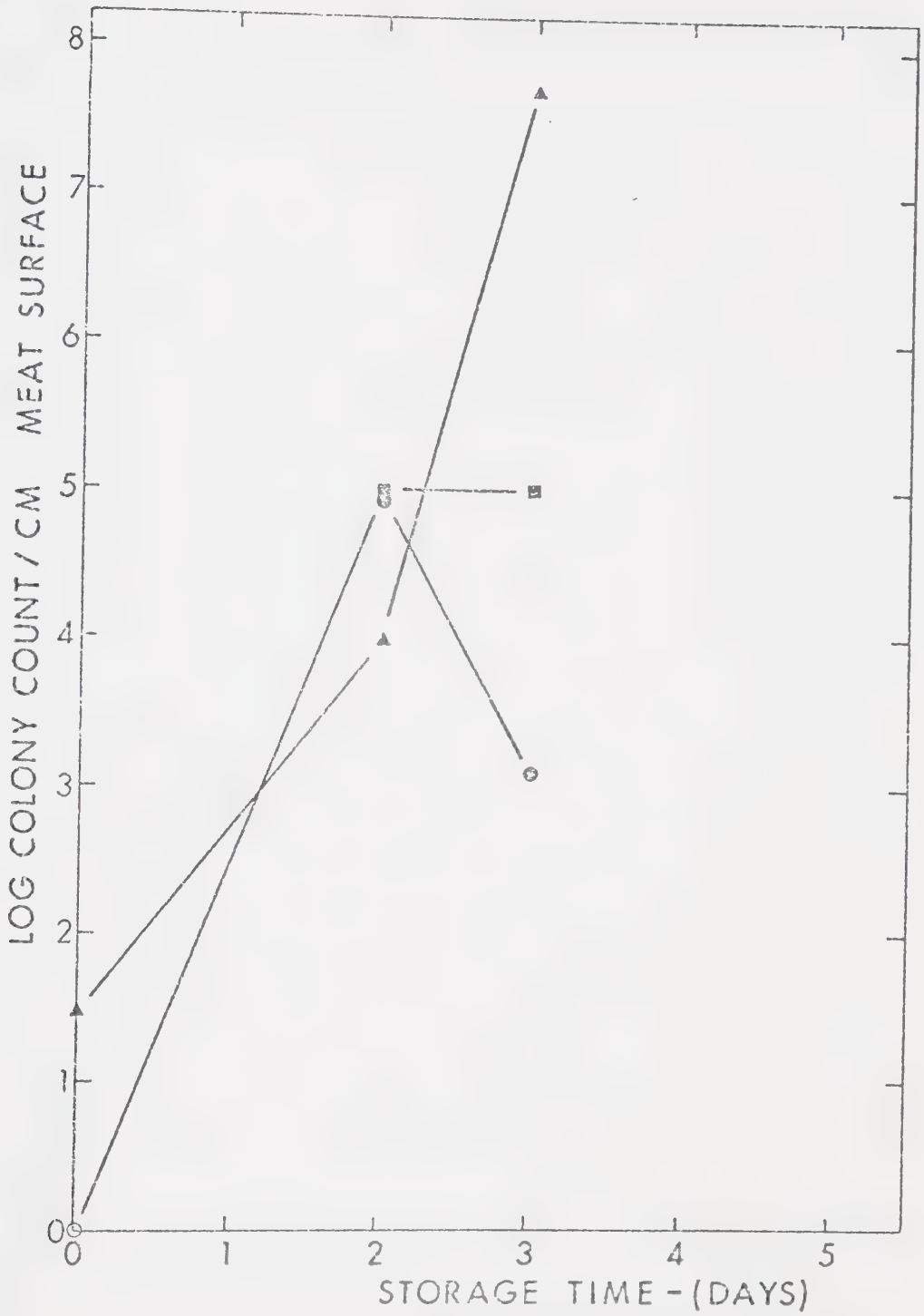

Fig. 36 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 12.5° and 85% RH after treatment with water sprays of various temperatures. TPC: O no treatment; ■ 20°; ◉ 53°: ▲ 70°. See Appendix XII A.

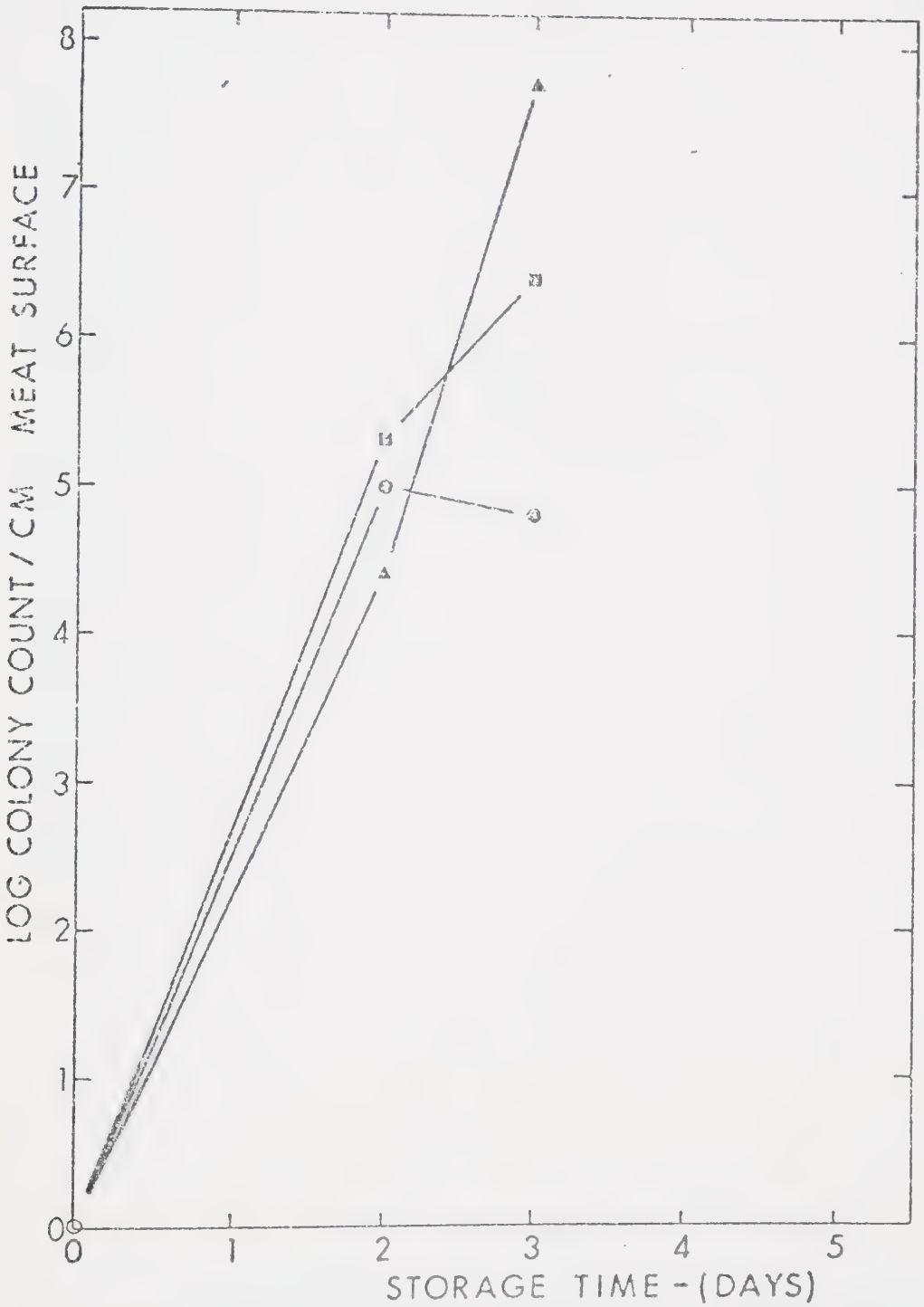

Fig. 37 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 12.5° and 85% RH after treatment with water sprays of various temperatures. PC: O no treatment; ■ 20°; ● 54°; ▲ 70°. See Appendix XII A.

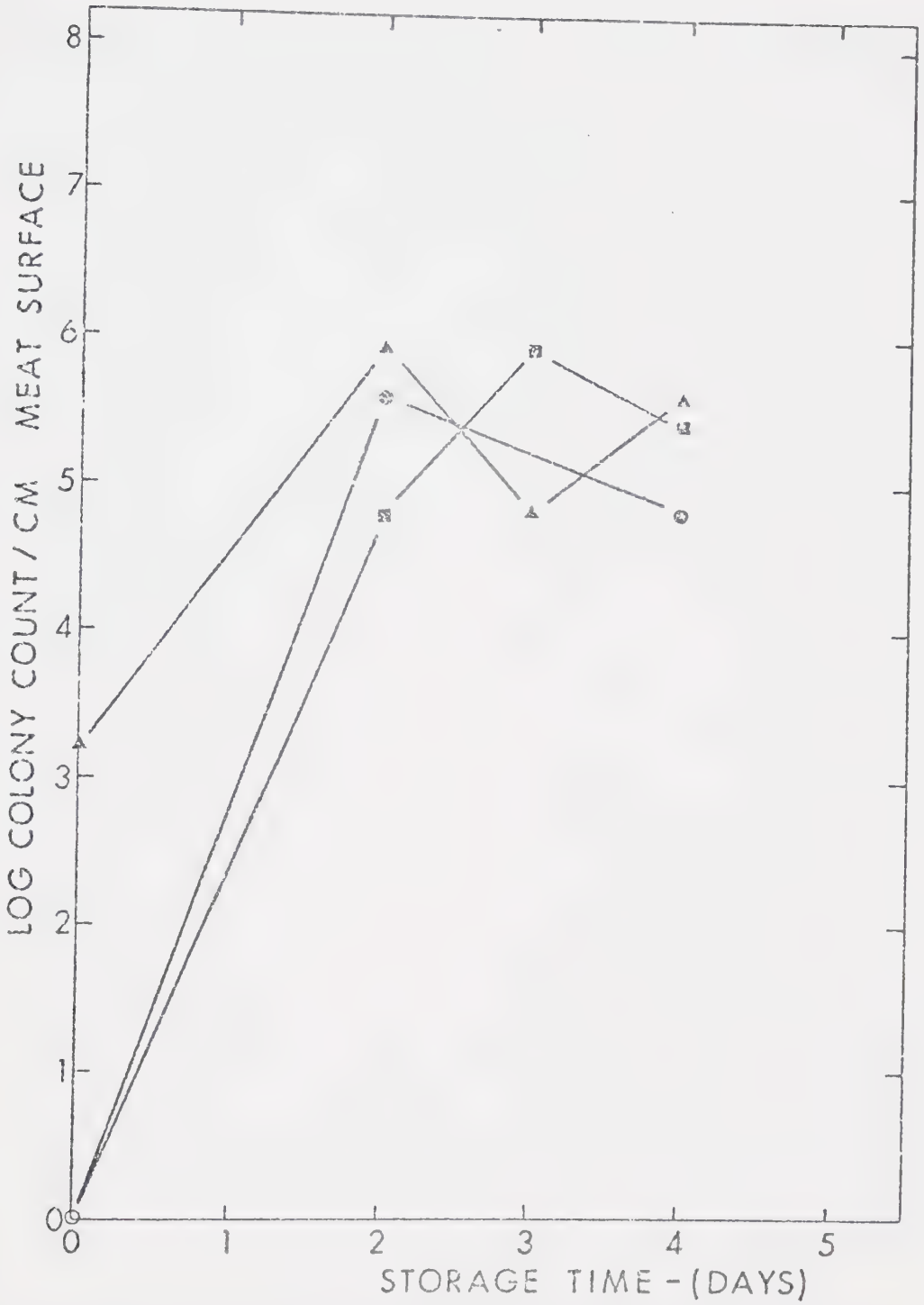

Fig. 38 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 12.5° and 85% RH after treatment with water sprays of various temperatures. TPC: O no treatment; ■ 18°; ● 53°; ▲ 70°. See Appendix XII B

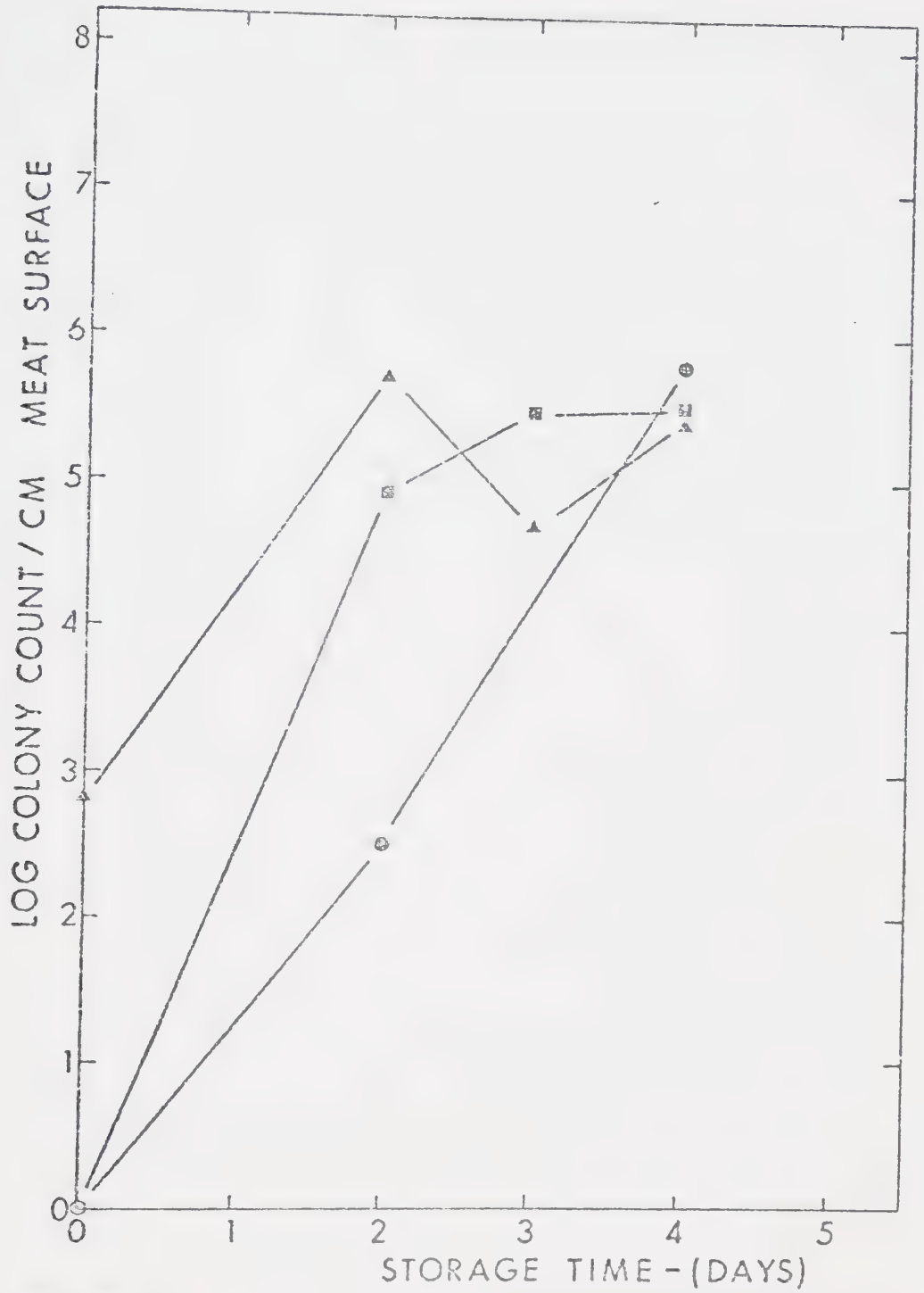

Fig. 39 The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 12.5° and 85% RH after treatment with water sprays of various temperatures. PC: O no treatment; ■ 18°; ● 53°; ▲ 70°. See Appendix XII B.

enrichment procedure should have given some positive results if there was heavy contamination with salmonellae. The results of Weissman and Carpenter (1968) might have been misleading because only two plants were examined in that study. The work of Hobbs and Wilson (1959) was reported from Britain where the climate is more humid than Alberta so the aging rooms in that country might be more favourable towards the development of salmonellae. Also Britain imports more meat from countries, such as Argentina, which have poor hygiene standards (Hobbs and Wilson, 1959). The results of this study indicated that the presence of salmonellae is not as prevalent a health hazard as might be expected on beef from the packinghouses examined in Edmonton.

CONCLUSIONS

One of the difficulties in exploratory work of this nature is the variation that can occur between duplicate samples. Normally when working with material which can give variable results steps are taken to replicate the testing and the results are statistically analysised to minimize the experimental error. However because of the time involved for a single determination extensive replication of experiments was impossible in this work. Statistics has therefore not been applied to evaluate the results. Accordingly it is not possible to be emphatic about any single result. However there are some trends that can be repeatly observed in different experiments, so some conclusions can be drawn from these findings. With these qualifications in mind the following conclusions are made.

(1) The results of this study indicated that 12.5^{o} and 15^{o} were probably too high for beef aging. Salmonella typhimurium grew on beef aged at 15^{o} and rapid increases in the numbers of spoilage micro-organisms were observed on samples stored at 12.5^{o}. The rate of microbial development was less at 10^{o} but some growth of Salmonella typhimurium was recorded. Further work would be necessary to insure that 10^{o} is a safe temperature at which to age beef.

(2) The effect of relative humidity was more pronounced when beef was aged at higher temperatures. Microbial development was more rapid on meat stored at 95% RH when the storage temperature was 12.5^{o} or 15^{o}. This trend was not evident at lower storage temperatures (4.4^{o} and 2^{o}).

Aging at low RH (40 or 1%) was not advantageous because microbial development was not markedly reduced and some discolouration resulted. This work indicated that there was little difference between aging at 75% or 85% RH with regards to microbial growth on the meat surfaces. General principles would suggest that moisture loss through evaporation and the resultant weight loss should be less at the higher RH. Accordingly a level of approximately 85% would seem to be the RH of choice.

(3) Attempts to reduce surface contamination with chlorine and hot water sprays gave anomalous results. Sprays containing 3 and 20 ppm chlorine had no effect in reducing the rate of microbial development. In one experiment 500 ppm chlorine did show inhibition of microbial growth but this was not observed in the other experiment. Hot water sprays also did not reduce the rate of bacterial development. Samples treated with water sprays at the highest temperature (70^{o}) actually had the highest counts/cm^2 at the end of the aging period. The reason for this is not clear.

(4) The rate of growth of Salmonella typhimurium was not great at 10^{o}. Salmonella counts increased a hundred fold on inoculated samples in one experiment while the naturally occuring contaminates increased by 10^9 when the meat was stored at 10^{o} for four days. No salmonellae were isolated from uninoculated meat samples of from carcass swabs in commercial packinghouses by the methods used in this work. The apparent low level of salmonella contamination in the packinghouses examined might be explained by the low temperatures and the low relative humidities used for aging.

(5) The bacteriological problem of greatest economic importance

when aging beef at high temperatures is the growth of spoilage micro-organisms of the Pseudomonas and Achromobacter genera. Many of these bacteria have growth optima close to the temperatures used in this work for high temperature aging. This research illustrated that the type and amount of natural contamination was important in determining the rate of meat spoilage. The presence of large numbers of psychrophilic organisms can result in rapid spoilage.

SUGGESTIONS FOR FUTURE WORK

(1) The use of disinfection treatments (heat and chemicals) in the water sprays should be further investigated. The volume and velocity of sprays may be important and sprays hotter than 70° might be beneficial. The lowest effective concentration of chlorine should be determined and the possible effect of chlorine on flavour should be studied. Possible synergistic effects of hot water and chlorine treatment should be investigated. Other types of disinfectants might prove useful.

(2) Results on experimental aging reported here involve only simulated laboratory trials. Aging an entire carcass is a much more complex situation. Trials with entire carcasses would be necessary before any high temperature aging process could be recommended for general use.

(3) Further studies on microbial growth in non-surface areas (in deep cut or in joints) would be necessary to insure that the aging process would be safe from a public health viewpoint.

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Appendix I The effect of various diluents on the survival of a diluted culture of Salmonella typhimurium stored at 24°.

Time in hr.	Count/ml (10^9)			
	Peptone H_2O (0.1%)	Peptone H_2O $MgSO_4$ (10^{-4}M)	K Phosphate Buffer pH 7	Ringer's sol. (1/4 strength)
0	2.74	2.62	2.97	3.27
1	2.47	2.34	1.96	2.73
2	2.41	2.20	1.16	1.97
3	1.98	1.97	0.36	2.40
4	1.94	1.97	0.08	1.81
5	2.12	2.03	0.00	2.01
6	2.73	2.46	0.01	2.01
7	3.77	4.35	0.01	2.27

Appendix II The development of micro-organisms on the surface of chuck steak stored at 15° and 95% relative humidity with and without Salmonella typhimurium inoculation.

Sampling Time-Day		Count/cm^2 of Meat Surface			
		Uninoculated		Inoculated	
		TPC	Salm. Count	TPC	Salm. Count
A					
	0	4.5×10^5	ND	1.2×10^6	1.1×10^6
	0	-	-	7.0×10^5	5.5×10^5
	1	2.3×10^7	ND	4.5×10^7	6.8×10^7
	1	-	-	7.7×10^7	2.7×10^7
B					
	0	9.8×10^5	ND	9.8×10^5	7.9×10^5
	0	-	-	1.1×10^6	1.0×10^6
	1	5.2×10^7	ND	1.6×10^8	2.1×10^7
	1	-	-	1.2×10^8	5.0×10^8

ND none detected

Appendix III The development of micro-organisms on the surface of chuck steak stored at 10° and 95% relative humidity with and without Salmonella typhimurium inoculation.

Sampling Time-Day	Count/cm^2 of Meat Surface			
	Uninoculated		Inoculated	
	TPC	Salm. Count	TPC	Salm. Count
0	6.8×10^3	ND	5.4×10^5	4.5×10^5
0	-	-	1.0×10^6	9.2×10^5
1	1.9×10^6	ND	6.4×10^7	1.5×10^6
1	-	-	9.1×10^7	1.3×10^8

ND none detected

Appendix IV The development of micro-organisms on the surface of flank steak obtained 3 hr. post-slaughter and stored at 10° and 95% relative humidity with and without Salmonella typhimurium inoculation.

Sampling Time-Day	Count/cm^2 of Meat Surface			
	Uninoculated		Inoculated	
	TPC	Salm. Count	TPC	Salm. Count
0	1.5×10^4	ND	3.0×10^5	3.0×10^5
0	-	-	4.6×10^5	8.9×10^4
1	1.5×10^4	ND	1.5×10^4	ND
1	-	-	1.0×10^5	3.0×10^4
4	3.0×10^9	ND	1.1×10^9	1.3×10^8
4	-	-	3.0×10^9	5.1×10^8
	TPC at 10°		TPC at 10°	
0	ND		ND	
0	-		ND	
1	3.0×10^4		ND	
1	-		ND	
4	3.0×10^9		8.6×10^8	
4	-		3.1×10^9	

ND none detected

Appendix V The development of micro-organisms on the surface of flank steak obtained 2 days post-slaughter and stored at 10° and 95% relative humidity with and without Salmonella typhimurium inoculation.

Sampling Time-Day	Count/cm^2 of Meat Surface			
	Uninoculated		Inoculated	
	TPC 37	TPC 10	TPC 37	TPC 10
0	4.3×10^3	ND	7.0×10^5	ND
0	-	-	6.7×10^5	ND
4	3.1×10^6	4.8×10^6	8.9×10^7	4.5×10^7
4	-	-	4.5×10^7	6.6×10^6
6	1.5×10^6	5.5×10^7	9.8×10^7	1.4×10^8
6	-	-	5.7×10^8	6.4×10^8

ND none detected

Appendix VI The relationship of aging time to the numbers of micro-organisms on the flank, mid rib and forward rib areas of beef carcasses stored under industrial conditions.

Carcass Number	Age in Days	Log. of Count/cm^2 of Meat Surface					
		TPC			PC		
		Flank	Mid-Rib	Forward Rib	Flank	Mid-Rib	Forward Rib
15	5	1.8195	2.6990	4.2553	1.9956	2.6335	4.1761
16	6	1.6990	*	2.0792	1.9956	*	2.4472
17	6	1.2304	2.5185	2.4472	1.8195	2.6990	2.6128
18	6	2.0792	2.1139	1.8195	2.2304	1.8195	2.0792
19	6	1.5185	2.7482	2.5798	1.6990	3.1139	2.3010
29	6	*	*	3.4314	*	*	4.8195
30	6	1.9956	*	*	*	*	*
6	7	3.9395	4.4771	2.7709	4.1139	4.6812	2.7482
7	7	4.8195	1.6990	2.4771	4.1139	1.8195	2.1139
14	7	2.6628	2.7709	2.1139	2.8976	2.9494	1.2304
1	8	3.8325	*	2.3222	4.9638	*	2.6335
2	8	2.5441	1.8195	1.8195	3.6812	1.9956	1.6990
3	8	1.2304	*	1.5185	1.5185	*	1.6990
4	8	3.9395	2.9638	5.7160	5.5185	5.1461	5.9956
5	8	2.5185	1.2041	2.1761	3.5798	3.0414	2.0792
8	8	2.7853	3.3010	3.7993	2.9590	3.4624	4.1461
9	8	3.1761	3.1461	3.3010	3.2041	3.0000	3.3979
10	8	1.2304	1.2304	*	2.3617	1.5185	1.2304
12	9	3.8808	4.3010	3.5185	3.7993	4.3010	3.5563
13	9	*	3.6021	4.0792	*	3.6128	4.0414
21	9	1.2304	2.0792	3.4472	*	*	4.4624
22	9	1.2304	5.0792	1.9191	*	4.9823	2.1761
25	9	*	5.4771	1.2304	*	5.5051	1.8195
28	10	4.4314	5.4771	5.9445	4.4624	5.4624	5.9956
11	12	2.2304	4.6628	5.0414	3.9638	5.3802	5.3222
23	13	*	4.8976	4.4472	*	5.9956	5.9956
24	13	*	5.9956	5.1461	3.2041	5.9956	5.9956
20	15	5.6990	5.7782	4.6990	5.7924	6.0414	4.8633
26	15	*	2.3617	*	*	2.4771	*
27	15	*	1.2304	3.6335	*	*	4.9494

* <33/cm^2

Appendix VII A The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 10° and various relative humidities.

Sampling Time-Day	Count/cm^2 of Meat Surface					
	95%		40%		<1%	
	TPC	PC	TPC	PC	TPC	PC
0	4.0×10^2*	9.2×10^2*				
2	3.3×10^6	9.0×10^5	1.6×10^3	1.1×10^6	1.3×10^6	1.1×10^6
2	3.3×10^6	9.0×10^5	4.5×10^3	9.0×10^5	3.9×10^6	9.0×10^5
3	7.2×10^7	7.8×10^7	2.6×10^6	8.1×10^7	-	-
3	1.7×10^8	1.5×10^8	3.9×10^7	1.2×10^7	-	-
4	2.7×10^8	2.9×10^8	-	-	7.8×10^7	8.1×10^7
4	2.5×10^8	3.9×10^8	-	-	6.9×10^6	1.1×10^7
5	-	-	2.7×10^6	9.6×10^7	-	-
5	-	-	4.5×10^5	5.7×10^8	-	-
6	-	-	-	-	8.7×10^7	9.6×10^7
6	-	-	-	-	3.3×10^8	5.7×10^8

* day 0 control

Appendix VII B The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 10° and at various relative humidities.

Sampling Time-Day	Count/cm^2 of Meat Surface					
	95%		40%		<1%	
	TPC	PC	TPC	PC	TPC	PC
0	ND*	ND*				
2	1.5×10^2	3.6×10^2	3.0×10^1	ND	2.1×10^3	1.9×10^3
2	ND	ND	6.9×10^2	2.2×10^3	6.0×10^1	9.0×10^1
3	8.9×10^5	1.0×10^6	ND	ND	-	-
3	4.2×10^3	2.0×10^4	2.0×10^4	6.3×10^3	-	-
4	2.7×10^5	1.1×10^6	-	-	ND	ND
4	2.6×10^7	4.3×10^7	-	-	ND	ND
5	-	-	1.2×10^4	4.4×10^4	-	-
5	-	-	6.0×10^3	1.1×10^4	-	-
6	-	-	-	-	1.1×10^4	1.1×10^4
6	-	-	-	-	6.9×10^2	6.0×10^2

* day 0 control

ND <$33/cm^2$

Appendix VIII The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 12.5° and at various relative humidities.

Sampling Time-Day	Count/cm^2 of Meat Surface					
	95%		85%		75%	
	TPC	PC	TPC	PC	TPC	PC
A						
0	6.9×10^2*	4.0×10^2*	* day 0 control			
0	1.2×10^3*	7.3×10^2*				
2	9.9×10^6	9.9×10^6	9.9×10^6	9.9×10^6	9.9×10^6	9.9×10^6
2	5.8×10^6	5.2×10^6	9.9×10^6	9.9×10^6	9.9×10^6	9.9×10^6
3	1.5×10^8	1.7×10^8	3.4×10^8	3.8×10^8	1.5×10^6	2.1×10^7
3	5.8×10^8	4.8×10^8	1.5×10^7	1.6×10^7	2.2×10^7	1.9×10^6
4	2.5×10^8	2.4×10^8	3.2×10^7	3.7×10^7	1.1×10^8	1.7×10^8
4	1.4×10^9	1.4×10^9	2.5×10^8	2.7×10^8	1.7×10^8	2.2×10^8
B						
0	1.7×10^2*	3.3×10^1*	* day 0 control			
0	1.4×10^3*	9.9×10^1*				
2	5.0×10^3	4.0×10^3	1.0×10^5	1.1×10^5	2.1×10^5	2.3×10^5
2	3.2×10^5	3.2×10^5	1.7×10^4	1.9×10^4	9.9×10^2	6.6×10^2
3	7.0×10^7	6.6×10^7	8.9×10^4	1.3×10^6	1.7×10^6	1.6×10^6
3	6.6×10^5	2.8×10^6	2.0×10^6	2.4×10^6	9.9×10^5	1.1×10^6
4	2.4×10^6	4.3×10^6	2.7×10^7	3.0×10^7	7.9×10^6	7.9×10^6
4	1.2×10^6	1.2×10^6	3.3×10^6	2.8×10^6	7.9×10^6	6.6×10^6

Appendix IX A The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 10° and at various relative humidities.

Sampling Time-Day	Count/cm^2 of Meat Surface					
	95%		85%		75%	
	TPC	PC	TPC	PC	TPC	PC
0	5.0×10^1*	ND*				
2	3.3×10^3	3.3×10^3	3.3×10^3	3.3×10^3	3.3×10^3	3.3×10^3
2	3.3×10^3	1.7×10^3	3.3×10^3	3.3×10^3	3.3×10^3	3.3×10^3
3	4.2×10^6	7.9×10^6	3.3×10^5	4.5×10^5	6.6×10^3	1.3×10^4
3	6.6×10^3	6.3×10^5	3.3×10^3	3.3×10^3	9.9×10^3	3.3×10^3
4	2.5×10^6	1.6×10^4	1.6×10^4	1.9×10^4	8.9×10^4	1.2×10^5
4	5.0×10^6	4.3×10^6	2.0×10^3	1.7×10^3	4.0×10^4	3.3×10^5
		PC @ 10°		PC @ 10°		PC @ 10°
0		ND*				
2		3.3×10^3		3.3×10^3		3.3×10^3
2		3.3×10^3		3.3×10^3		1.2×10^4
3		1.3×10^7		4.0×10^5		2.3×10^4
3		9.6×10^5		3.3×10^3		3.3×10^3
4		8.9×10^6		1.3×10^4		1.1×10^5
4		4.6×10^6		5.0×10^2		1.7×10^6

* Day 0 conrtol

ND <$33/cm^2$

Appendix IX B The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 10° and at various relative humidities.

Sampling Time-Day	Count/cm^2 of Meat Surface					
	95%		85%		75%	
	TPC	PC	TPC	PC	TPC	PC
0	5.9×10^3*	9.9×10^3*				
2	7.9×10^6	9.9×10^6	2.6×10^6	9.9×10^6	8.3×10^5	2.7×10^6
2	2.0×10^6	9.9×10^6	1.8×10^6	1.7×10^6	4.2×10^6	9.9×10^6
3	8.3×10^7	2.4×10^8	6.6×10^6	2.4×10^7	4.1×10^6	spoiled
3	1.3×10^7	7.6×10^7	6.6×10^7	2.3×10^7	1.7×10^7	spoiled
4	8.9×10^7	1.3×10^8	2.6×10^7	3.1×10^7	3.4×10^7	4.3×10^7
4	1.5×10^8	2.7×10^8	3.2×10^7	1.1×10^8	4.8×10^7	4.0×10^7
		PC @ 10°		PC @ 10°		PC @ 10°
0		5.6×10^2*				
2		9.9×10^6		9.9×10^6		3.0×10^6
2		4.3×10^6		1.7×10^6		9.9×10^6
3		spoiled		spoiled		1.1×10^7
3		spoiled		7.3×10^8		3.2×10^7
4		1.5×10^8		2.8×10^7		3.4×10^7
4		2.2×10^8		9.6×10^7		7.6×10^7

* Day 0 control

Appendix X The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at (A) 4.4° and (B) 2° and various relative humidities.

Sampling Time-Day		Count/cm^2 of Meat Surface					
		95%		85%		75%	
		TPC	PC	TPC	PC	TPC	PC
A							
	0	2.5×10^2*	8.3×10^1*				
	0	8.3×10^1*	5.0×10^1*				
	2	1.3×10^3	6.6×10^2	1.7×10^3	3.4×10^3	2.5×10^3	9.2×10^3
	2	3.0×10^2	4.0×10^2	1.6×10^3	2.4×10^3	3.0×10^2	9.9×10^1
	4	5.3×10^3	8.6×10^3	1.0×10^4	2.3×10^4	9.6×10^5	8.3×10^5
	4	8.6×10^4	1.4×10^5	1.7×10^5	2.2×10^5	2.3×10^4	4.3×10^4
	8	7.6×10^6	5.3×10^6	6.3×10^6	1.0×10^7	3.6×10^6	3.3×10^6
	8	6.3×10^6	6.3×10^6	5.3×10^6	1.1×10^7	1.1×10^7	9.6×10^6
B							
	0	4.6×10^2*	1.7×10^2*				
	0	2.8×10^3*	1.3×10^3*				
	4	3.3×10^2	ND	5.0×10^2	7.6×10^2	3.1×10^3	6.4×10^3
	4	5.6×10^2	7.9×10^2	4.5×10^3	3.0×10^3	6.9×10^2	3.3×10^2
	6	1.3×10^3	5.3×10^5	9.9×10^6	9.9×10^6	6.1×10^4	8.8×10^4
	6	2.1×10^6	8.3×10^6	4.4×10^4	1.2×10^5	2.8×10^4	7.3×10^4

* Day 0 control

ND <33/cm^2

Appendix XI The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 12.5° and 85% RH after treatment with sprays of various chlorine concentrations. * untreated day 0 control ** treated day 0 control ND less than 33/cm^2

Sampling Time-Day	Count/cm^2 of Meat Surface					
	3 ppm		20 ppm		500 ppm	
	TPC	PC	TPC	PC	TPC	PC
A						
0	3.0×10^2*	9.9×10^1*				
0**	9.9×10^1	ND	3.3×10^1	1.7×10^1	3.3×10^1	3.3×10^1
2	3.0×10^5	1.2×10^6	1.1×10^5	1.9×10^5	4.3×10^3	6.6×10^3
2	1.3×10^5	1.9×10^5	6.9×10^5	5.6×10^5	2.8×10^4	3.0×10^4
3	2.4×10^6	2.7×10^6	2.2×10^7	2.0×10^7	ND	ND
3	1.8×10^6	3.9×10^6	5.0×10^7	5.7×10^7	7.6×10^5	7.9×10^5
4	1.7×10^7	4.3×10^6	4.6×10^6	4.0×10^6	2.3×10^6	5.9×10^6
4	3.8×10^6	5.1×10^6	1.8×10^8	1.5×10^8	2.2×10^5	3.1×10^5
B						
0	ND*	ND*				
0**	6.6×10^1	3.3×10^1	ND	ND	ND	ND
2	1.2×10^4	1.1×10^4	1.3×10^3	6.6×10^2	ND	ND
2	4.0×10^4	3.6×10^4	9.9×10^2	ND	ND	ND
3	1.4×10^6	1.6×10^6	6.6×10^2	ND	5.9×10^3	5.0×10^3
3	1.4×10^6	1.4×10^6	6.3×10^5	5.3×10^5	ND	ND
4	1.7×10^6	1.8×10^6	3.8×10^5	3.0×10^5	6.9×10^3	6.3×10^3
4	4.7×10^5	5.0×10^5	1.3×10^5	1.0×10^6	ND	ND

Appendix XII The development of micro-organisms on the surface of flank steak obtained 24 hr. post-slaughter and stored at 12.5° and 85% RH after treatment with water sprays of various temperatures.

Sampling		Count/cm^2 of Meat Surface					
	A	20°		54°		70°	
	B	18°		53°		70°	
		TPC	PC	TPC	PC	TPC	PC
A							
	0	ND*	ND*				
	0√	ND	ND	ND	ND	3.3×10^1	ND
	2	1.5×10^5	3.3×10^5	ND	ND	2.4×10^4	3.6×10^4
	2	5.3×10^4	9.2×10^4	1.8×10^5	2.0×10^5	ND	ND
	3	9.9×10^4	1.2×10^6	2.6×10^3	1.5×10^5	1.8×10^7	2.0×10^7
	3	1.2×10^5	4.5×10^6	ND	ND	9.9×10^7	9.9×10^7
	4	all samples spoiled					
B							
	0	ND*	ND*				
	0√	ND	ND	ND	ND	1.8×10^3	7.3×10^2
	2	1.1×10^5	1.5×10^5	2.3×10^4	2.2×10^4	1.3×10^6	7.9×10^5
	2	2.7×10^4	3.2×10^4	8.3×10^6	6.8×10^6	4.6×10^5	4.6×10^5
	3	1.3×10^6	2.0×10^5	samples spoiled		1.2×10^5	8.9×10^4
	3	3.9×10^5	5.0×10^5	samples spoiled		2.3×10^4	1.3×10^4
	4	6.6×10^4	7.9×10^4	8.3×10^5	7.3×10^5	3.3×10^3	ND
	4	5.6×10^5	6.6×10^5	5.3×10^5	7.3×10^5	8.6×10^5	5.9×10^5

* untreated day 0 control
√ treated day 0 controls ND $<33/cm^2$